TROPHIC TRANSFER OF ORGANOCHLORINES IN A SALT MARSH ESTUARY

by

JEREMY S. SMITH

(Under the direction of Aaron T. Fisk)

ABSTRACT

Trophic magnification factors (TMF) have been used to quantify the movement of organchlorines (OCs) in food webs using isotopes of stable nitrogen ($\delta^{15}N$) in marine and freshwater systems, but have not been applied to estuarine food webs. To address this data gap, TMFs and food web structure were studied in a salt marsh biota from a contaminated estuary in the southeastern U.S. (Brunswick GA). $\delta^{13}C$ and $\delta^{34}S$ were also used to characterize the food web and trophic transfer of OCs. Based on $\delta^{13}C$ and $\delta^{34}S$ values, seston and *S. alterniflora* were the major contributors to secondary production in the estuary, with little influence from upland plants. Only polychlorinated biphenyls (PCBs) 187, -202, -180, and -199 had a significant relationship with trophic position, with TMFs of 1.87, 2.38, 2.47, and 3.61, respectively. There were no significant relationships with toxaphene and trophic position. $\delta^{13}C$ was found to be correlated with both trophic position and $\delta^{34}S$, so multiple regression was only used to determine if $\delta^{34}S$ values reduced variability associated with calculated TMFs. Values $\delta^{34}S$ only decreased variability in trophic transfer of PCB congeners by ~1-2 %. There were no significant multiple regressions for any toxapahene congener. In general, more studies of this type are required to understand the dynamics of OCs in estuaries.

Keywords: organochlorines, estuaries, trophic transfer, multiple stable isotopes

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DEDICATION

I would like to dedicate this thesis to my parents for their endless support, encouragement, inspiration and love. They have taught me more than they will ever know.

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CHAPTER 1

INTRODUCTION

Organochlorines (OC) are a group of compounds used in industry and agriculture commonlyreferred to as persistent organic pollutants (POP). These compounds are characterized by their low water solubility, resistance to biodegradation, and potential to accumulate in fat (de March et al. 1998). Many OCs also have the potential for long-range transport in the environment, and are found in distant locations from sources (Wania and Mackay 1999). As a result, these compounds remain in the environment for long periods of time and can reach high, and sometimes toxic, levels in fish and wildlife. Even though many OCs that have been out of use for decades (e.g. PCBs, DDT, toxaphene), they are not only still present, but have also become ubiquitous in the environment. Understanding the transport and fate of OCs in the environment is an essential part of determining their potential risks to humans and wildlife. OCs that have an extremely low water solubility (i.e. hydrophopic), and are recalcitrant tend to increase in concentration with increasing trophic level (Kidd et al. 1995, Fisk et al. 2001, Ruus et al. 2002). Trophic magnification factors (TMF) have been used in recent years to quantify movement of OCs from one trophic level to the next, often called *trophic transfer* (Fisk et al. 2001, Ruus et al. 2002). TMFs are calculated by comparing the OC concentration in an individual with its trophic position (Fisk et al. 2001, Ruus et al. 2002, Hop et al. 2002), which determined from stable isotopes of nitrogen ($\delta^{15}N$), that also increases with trophic level (Minagawa and Wada 1984). The benefit of using TMFs is that it incorporates the entire food web rather than specific predator-prey interactions (Borgå et al. 2004). TMFs have become

important tools in determining fate of OCs in marine and freshwater systems, but to date have not been readily used to assess trophic transfer of OCs in estuarine ecosystems.

Estuaries are multifaceted systems because they have characteristics that are a combination of freshwater and marine systems. Tides can cause frequent and sometimes extreme changes in temperature, pH, salinity, and dissolved oxygen in estuaries (Long and Mason 1983, Packham and Willis 1997). Estuaries are also very productive systems with allochthonous inputs from upstream, terrestrial systems and autochthonous inputs from algal and bacterial production (Long and Mason 1983). Many fish species use estuaries for at least some of their life cycles, especially juveniles, because there are ample food resources and refuge from predators, and have been termed the "nurseries of the oceans" (Minello et al. 2003). Thus it is important to understand the impact and fate of OC contaminant in these intricate systems. However, the complexities of food web interactions in estuaries also make it difficult to assess trophic transfer of OCs.

To assess trophic transfer of OCs in estuaries it is necessary to understand trophic relationships and linkages associated with the food web. Stable isotopes of carbon and sulfur ($\delta^{13}C$, $\delta^{34}S$) are becoming increasingly useful tools in understanding trophic interactions in aquatic food webs, including estuaries. Many studies have used these two isotopes in trophic ecology to determine sources of primary production (Peterson et al. 1986, Peterson and Howarth 1987, Hsieh et al. 2002) and contribution of producers that support secondary production (Peterson and Howarth 1987, Kwak and Zedler 1997, MacAvoy et al. 2000, Currin et al. 2003). Using information derived from multiple stable isotopes ($\delta^{13}C$, $\delta^{34}S$, $\delta^{15}N$) may increase the ability to assess trophic transfer of OCs in estuarine ecosystems.

The goal of this thesis is to understand the factors that influence trophic transfer of OCs in a southeastern U.S. estuary, near Brunwick, GA, that is heavily contaminated with PCBs and toxpahene. This will be accomplished by first characterizing the estuarine food web using multiple stable isotopes (Chapter 2) and then using that information to assess trophic transfer of PCBs and toxaphene (Chapter 3). Trophic position has been shown to be a means to quantify trophic transfer (Fisk et al. 2001, Ruus et al. 2002), but to out knowledge has never been attempted in an estuary. This introductory chapter will review the basics of salt marsh ecology and how stable isotopes are utilized in trophic ecology. The chemical properties of major contaminants in the estuary (i.e. PCBs and toxaphene) and means of quantifying trophic transfer of OCs will also be discussed in this section.

1.1 Salt Marsh Estuaries

Salt marshes are a major constituent of the estuaries in the southeastern United States. By definition salt marshes are "areas of alluvial or peat deposits, colonized by herbaceous and small shrubby terrestrial vascular plants, almost permanently wet and frequently inundated with saline waters" (Long and Mason 1983). In Georgia alone, there are over 1500km^2 of salt marsh (GA DNR 2005), which are complex ecosystems and subject to a wide variety of physical, chemical, and biological interactions.

Along the Georgia coast, salt marshes are influenced by tides that can reach up to 3 m in vertical height twice daily (Long and Mason 1983). These tides create tidal drainage channels that are commonly known as tidal creeks and are abundant along the Georgia coast. Tidal creeks appear similar to other creeks or rivers; however, they are very different hydrologically. Water flow in tidal creeks is in two directions, and middle marsh areas reach bankful conditions

approximately 360 times in a year as opposed to once every two or three years in many rivers (Leeks 1979).

Where drainage is impeded in the marsh, tides contribute to waterlogging of soils, the primary result of which is the development of anoxia (Armstrong 1976). Under anaerobic conditions microbes make use of other electron receptors other than oxygen. As a consequence many compounds are chemically reduced, which results in an overall lowering of the oxidation-reduction potential of the soils (Armstrong 1976). A common characteristic of these soils is their grey or black coloration, which is due to metal precipitation by sulfides produced by sulfur-reducing bacteria (Long and Mason 1983).

Georgia salt marshes are known to be extremely productive systems (Mendelson and Morris 2000). *Spartina alterniflora*, the dominant plant species in the marsh, is thought to contribute greatly to the productivity of these systems (Long and Mason 1983, Mendelson and Morris 2000) and the major contributor of carbon (Wiegert 1979). Haines (1977, 1979) argued that algal production within salt marshes had been underestimated in previous studies and was also a major contributor to carbon in these systems. Elucidating sources of organic matter and fate in estuaries has been a topic of great interest (Haines 1976, Peterson et al. 1980, Sherr 1982, Peterson and Howarth 1987, Kwak and Zedler 1997, Hsieh et al. 2002, Chanton and Lewis 2002, Goñi et al 2003, Kaldy et al. 2005). Past studies have attempted to use stable isotopes to solve these unanswered questions, but they have relied upon single isotopic tracers to identify sources of primary production in estuaries (Haines 1976, Sherr 1982, Goñi et al 2003).

1.2 Food Webs and Stable Isotopes

Food webs describe the relationships and trophic hierarchy between predator and prey in communities and ecosystems (Pimm 1991). Characterizing food webs is a basic step in

understanding the structure of ecological communities and developing population and community models and have become important themes in current ecological research. Pathways of energy transfer in ecosystem can also be deduced from food webs (Schonley and Cohen 1991, Link 2002). Despite the importance of food webs, they still remain difficult to quantify due to their dynamics, complexity, and sensitivity to environmental factors (Vander Zanden et al. 1999).

Stomach content analysis has been a widely used method for constructing trophic relationships (Cortes 1999), because it allows the investigator to know the diet items of consumers. However, it is subject to many potential problems such as: empty stomachs (Renones et al. 2002), unidentifiable material (Pinnegar et al. 2001), and differences in digestibility of prey (Pinnegar and Polunin 2000). Often, the organism must be sacrificed to analyze stomach contents; which can be an issue for rare or threatened species. Furthermore, stomach content analysis provides no temporal data, but only a snapshot of an organism's diet (Pinnegar and Polunin 2000).

The analysis of natural stable isotope abundance has become an increasingly important tool in food web studies. The two most common stable isotopes used in food web studies are those of carbon (^{13}C) and nitrogen (^{15}N). Stable isotopes can often provide more insight into the diet and feeding behavior of organisms than stomach content analysis, because it is a measure of what is actually incorporated into the individual (Peterson and Fry 1987). However, stable isotope analysis is not without its limitations. Using single isotopic tracers makes it difficult to determine specific feeding relationships, especially when various diet items have the same isotopic signature. In general, isotopes can be used to trace organic matter when there are n isotopes used and there are $n + 1$ diet items (Phillips and Gregg 2003). This, however, is

typically not the case in most aquatic systems. Analysis of multiple stable isotopes (^{13}C, ^{15}N, and ^{34}S), therefore, allows better resolution of food web structure, but is still limited when there are several potential diet items for an organism.

Stable isotopes of elements (e.g. C, N, S, O, H) vary only in the number of neutrons in the nucleus, which give them different masses. Isotopes with more neutrons are considered heavy, whereas those with less are regarded as light. Isotopes described as being stable implies that they do not undergo radioactive decay. Stable isotopes partake in all chemical reactions associated with that element, but differences in mass cause the different isotopes to react at different rates. These differences in the rate of chemical reactions result in changes in the relative abundance of stable isotopes in different compartments of the environment. Changes in isotope abundance are often predictable and one form is usually more abundant, making the more rare form a well-suited ecological tracer (Peterson and Fry 1987).

Using stable isotopes as food web tracers relies on comparing changes in isotopic ratios of diet items to those in the consumer. These changes in isotopic ratios, or fractionation, occur on a small scale, and to make these differences more discernable, delta (δ) notation is commonly used. The ratios of heavy to light isotopes ($^{13}C/^{12}C$, $^{34}S/^{32}S$, and $^{15}N/^{14}N$) are most often expressed as part per mil (‰) deviation from a standard and δ- notation according to:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where X is ^{13}C, ^{34}S, or ^{15}N and R is the ratio of $^{13}C/^{12}C$, $^{34}S/^{32}S$, and $^{15}N/^{14}N$, respectively. $R_{standard}$ values are based on PeeDee Belemnite for ^{13}C, Diablo Canyon meteorite for ^{34}S, and atmospheric N_2 (air) for ^{15}N. When the δX of an organism is greater than that of the standard, it is said to be enriched, and depleted if it is less than that of the standard.

1.2.1 CARBON ISOTOPES

Differences in $\delta^{13}C$ can identify variations in sources of primary production supporting higher trophic levels, because it is conserved, meaning there is little fractionation of $\delta^{13}C$ from diet to consumers (Haines and Montague 1979, Peterson 1999). Values of $\delta^{13}C$ can separate primary producers because different modes of photosynthesis give plants unique isotopic signatures. Plants with the Calvin pathway (C_3) of photosynthesis discriminate against isotopically heavy CO_2, and thus have a high degree of fractionation of atmospheric CO_2, giving them a lighter isotopic signature averaging -28 ‰ (Troughton et al. 1974). C_4 plants do not exhibit as much discrimination as C_3 plants and are more enriched in ^{13}C with $\delta^{13}C$ values of -13‰ (Peterson and Fry 1987). Phytoplankton and dinoflagellates have intermediate $\delta^{13}C$ values of -20 to -22 ‰ due to the uptake of inorganic carbon from seawater (Haines 1976, Haines and Montague 1979, Peterson and Fry 1987). Carbon isotope values are also affected by plant habitat. France (1995) found that benthic aquatic plants tended to be more enriched in ^{13}C than pelagic plants. The stagnant boundary layer that surrounds benthic plants limits the rate of carbon (CO_2, HCO_3^-) diffusion and uptake, which leads to uptake of normally discriminated ^{13}C resulting in higher $\delta^{13}C$ signatures.

In southeastern U.S. estuaries, potential sources of organic matter are upland plants, plankton, and *Spartina alterniflora* (Peterson and Howarth 1987), all of which have different $\delta^{13}C$ values. Unlike many upland plants, *S. alterniflora* has the C_4 pathway of photosynthesis, and thus exhibits less discrimination against ^{13}C and is typically more enriched in ^{13}C, and has a more positive $\delta^{13}C$ value.

1.2.2 Sulfur Isotopes

Sulfur stable isotopes ($\delta^{34}S$) are also important tracers of organic matter sources, especially in estuarine food webs (Kwak and Zedler 1997, MacAvoy et al. 2000, Hsieh et al. 2002, Connolly et al. 2004). Like ^{13}C, there is very little fractionation from diet to consumer (Peterson et al. 1985). Marine plankton acquire sulfur from sulfates from the water column and typically have $\delta^{34}S$ values of about +20 ‰ (Chanton and Lewis 1999). *S. alterniflora* is isotopically lighter in ^{34}S (+0.9‰) due to uptake of sulfur-depleted sulfides in sediments (Peterson and Howarth 1987). Upland plants rely on sulfates from precipitation and have $\delta^{34}S$ ranging between +2 to +8‰ (Nriagu and Coker 1970). $^{34}S/^{32}S$ ratios are independent of carbon isotopic distribution, and ^{34}S is frequently used when carbon isotopes cannot unambiguously differentiate sources of primary production in estuarine food webs (Peterson and Howarth 1987, Kwak and Zedler 1997, Hsieh et al. 2002, Chanton and Lewis 2002, Currin et al. 2003). Hence, $\delta^{34}S$ can be used to distinguish between marine and freshwater sources of organic matter.

Using $\delta^{13}C$ and $\delta^{34}S$ values, organic matter originating from plankton, *S. alterniflora*, and upland plants can be separated (Peterson and Howarth 1987), and has been shown to be the best combination of isotopes to separate producers in estuarine ecosystems (Connolly et al. 2004).

1.2.3 Nitrogen Isotopes

The concept of trophic levels was introduced by Lindeman (1942), which represent progressive energy relationships in an ecosystem. However, discrete trophic levels did not take into account that organisms feed on a variety food sources from different trophic levels. Fractional trophic levels were introduced by Odum and Heald (1975) and are now considered estimates of trophic position (Vander Zanden et al. 1997).

Values of $\delta^{15}N$ can be useful in assessing trophic position of an organism within an aquatic food web (Vander Zanden et al. 1999). The ratio of heavier to lighter isotopes of nitrogen ($^{15}N/^{14}N$), relative to a standard, reported as $\delta^{15}N$, progressively increases with successive trophic levels. During nitrogenous excretion the lighter isotope is more easily removed from the body, whereas the heavier isotope is retained in body tissues (Peterson 1999). Studies have used an enrichment of 2 - 3.8 ‰ in $\delta^{15}N$ values from diet to consumer to represent one trophic level, termed an enrichment factor (Minigawa and Wada 1984). Comparing the $\delta^{15}N$ value of a consumer to a proper baseline $\delta^{15}N$ value provides a means to quantify its trophic position. The relative trophic position for consumers can be determined using the following relationship:

$$TP_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/3.4 + 2 \quad (2)$$

where $TP_{consumer}$ is the trophic level of the organism, $\delta^{15}N_{baseline}$ is the $\delta^{15}N$ values of the base of the food web, and 3.4 is the isotopic enrichment of $\delta^{15}N$ per trophic level (Hobson et al. 2002). Using $\delta^{15}N$ also takes into account omnivory in consumers rather than relying on specific predator-prey interactions (Vander Zanden et al. 1999), and provides a continuous base to determine trophic position.

1.3 Persistent Organic Pollutants

Persistent organic pollutants (POPs) include a variety of anthropogenic contaminants that are characterized by having low solubility in water, an affinity for lipids, and resistance to biodegradation (de March et al. 1998). Most POPs are halogenated hydrocarbons, in particular chlorine, which are often termed organochlorines (OC). Although many OCs, such as PCBs and toxaphene, have been banned in many countries, they continue to be a problem in the environment, in a large part because of their persistence.

A major concern about OCs in the environment is their potential to bioaccumulate. These compounds can accumulate in fatty tissues and are toxic to non-target organisms (de March et al. 1998). Compounds such as polychlorinated biphenyls (PCB) and organochlorine pesticides (toxaphene) have been shown to increase with increasing trophic level (Hobson et al. 2002, Ruus et al. 2003, Fisk et al. 2003), termed biomagnification. Elevated concentrations of these compounds could cause harm to higher trophic predators. For example, egg shell thinning in bald eagles, which decimated North American populations in the 1960s and 70s (Wiemeyer et al. 1993), was due to biomagnification of DDT in bald eagles from fish prey (Glaser and Connolly 2002). In fact, many states in the U.S. have consumption guidelines that limit consumption of fish due to high OC concentrations. OCs such as PCBs and the pesticide toxaphene are considered major global contaminants, including the Arctic (de March et al. 1998).

1.3.1 POLYCHLORINATED BIPHENYLS (PCBS)

There are a total of 209 polychlorinated biphenyl (PCB) congeners having from one to ten chlorines, of which about 100 are found in the environment (Figure 1.1). Physical properties and biological activity of PCB congeners vary with the number and position of chlorines on the biphenyl rings (Mackay et al 1992). Toxicity of PCB is linked to the position of chlorine on the biphenyl ring (Safe 1993). PCBs can exert a wide variety of toxic effects including liver toxicity, porphyria, immunosuppression, reproductive and developmental toxicity, cancer, and endocrine disruption (Faroon and Olson 2000). The most biologically active congeners are those with 3, 3', 4, 4' (PCBs 77, 126, 169) or 2, 3, 3', 4, 4'(PCBs 118, 105) chlorine substitutions (Ahlborg et al 1992, 1994). Increasing chlorination results in lower water solubility, greater persistence (Mackay et al. 1992), and generally greater bioaccumulation. PCBs, especially congeners

without adjacent unsubstituted positions on the biphenyl ring are not easily degraded in the environment. Half-lives of PCBs in air range from a few weeks to 2 years.

PCBs had a variety of uses from electric transformers and capacitors to inks and paints due to their stability and resistance to heat. PCBs were initially produced in 1929 by Monsanto Chemical Corporation under various tradenames such as: Aroclor, Clophen, and Phenoclor. Use was particularly high in the northern hemisphere, and the total estimated production of PCBs in the United States was 700,000 tons. Production of PCBs was banned in the United States in the 1970s (Faroon and Olson 2000), but use may continue in other countries (de March et al. 1998, Muir et al. 2000). Because of their unique chemical properties (hydrophobic, persistent, and recalcitrant) and extensive use in the past, PCBs have become ubiquitous and major global contaminants.

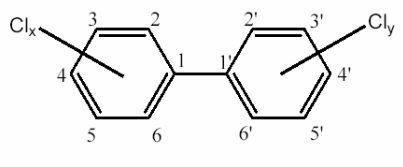

Figure 1.1. General structure of a polychlorinated biphenyl.

PCBs were produced in mixtures such as Aroclor 1242, 1250, 1262, and 1268. Aroclor nomenclature is decided based on the number of carbons in the molecule and percent chlorination of the mixture. For example, Aroclor 1242 has 12 carbon atoms and is 42 % chlorine by weight. Aside from the mixture Aroclor 1268, most other technical mixtures were used globally. Aroclor 1268 consists mainly of congeners having between 6 and 10 chlorine atoms and have been described as "superhydrophobic", having log octanol-water coeffients (log K_{ow}) ranging from 6.73 to 9.60 (Woodburn et al. 1984, Hawker and Connell 1988). Kannan et al. (1998) found that the congener profiles in sediment from salt marsh contaminated with

Aroclor 1268 resembled that of the technical mixture indicating that this particular mixture is highly stable in the environment. Biota from this area has also been shown to have PCB profiles comparable to Aroclor 1268 (Maruya and Lee 1998a, 1998b), in which case hepta-, octa-, and nonachlorobiphenyls made up 85-95% of the total PCB concentrations.

1.3.2 TOXAPHENE

Toxaphene (Figure 1.2) was once widely used as an agricultural pesticide and piscicide in much of North America and other parts of the world. Total production of toxaphene in the United States was 5 x 10^6 tons and peak usage was in 1974 when 2/3 of the production was applied in the southern states for cotton crops (Voldner and Li 1995). Other uses included treating lakes in the US and Canada to eradicate non-game fish (Miskimmin and Schindler 1994). Toxaphene was banned in the US in 1982, but stores were available for use until 1986. Since being banned, large reservoirs of toxaphene still endure, and volitilization from these reservoirs are thought to maintain relatively high atmospheric levels, allowing long-range transport (Harner et al. 1999). This has led to toxaphene and its residues to become ubiquitous and among the major global pollutants, even in the Arctic (Bidleman et al. 1993).

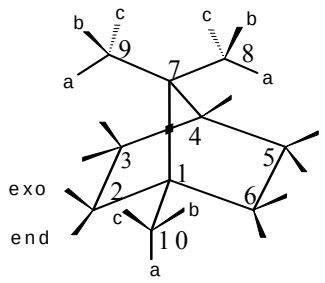

Figure 1.2. A bornane skeleton. The primary backbone of polychlorinated monoterpenes in technical toxaphene.

Technical toxaphene is an organochlorine created by a process involving chlorination of camphene until exhaustion. The result is a combination of polychlorinated bornanes, camphenes, and camphadienes averaging about 70% chlorine by weight (Saleh 1991). The technical mixture contains approximately 200 different components (Saleh 1991, de Geus 1999), with the most abundant homologues being hexachloro to decachloro compounds (Figure 1.2)

The physicochemical properties of toxaphene congeners are dependent upon the number and positioning of chlorine atoms on the bornane skeleton (Figure 1.2), which in turn determines the fate and behavior of congeners in the environment. Toxaphen log K_{ow}s range from 4.77 ± 0.076 to 6.64 ± 0.74 for 36 congeners (Fisk et al. 1999), which are in the range associated with OCs that biomagnify (Hoekstra et al. 2002, Fisk et al. 1998). The distribution of individual congeners found in environmental samples is often different from that of the technical mixture (Miskimmin et al. 1995, Stern et al. 1996, Whittle et al. 2000, Maruya et al. 2000, Muir et al. 2004). Several biotic and abiotic processes in the environment produce a toxaphene profile that consists of lower chlorinated congeners (Braekevelt et al. 2001, Maruya et al. 2001). Vetter and Maruya (2000), Vetter et al. (2001) and Maruya et al. (2001) found that the most prevalent congeners in biota were 2-exo, 3-endo, 6-exo, 8, 9, 10-hexachlorobornane (B6-923, Hx Sed) and 2-endo, 3-exo, 5-endo, 6-exo, 8, 9, 10-heptachlorobornane (B7-1001, Hp-Sed). These two congeners were also the most abundant in sediments from toxaphene-treated lakes (Stern et al 1996). Since these two congeners are not a major constituent of the technical mixture, it is believed that they are dead-end metabolites due to reductive dechlorination of highly chlorinated congeners in anoxic sediments (Stern et al 1996, Ruppe et al. 2003, 2004). Other important congeners found in biota and sediments are 2-endo, 3-exo, 5-endo, 6-exo, 8, 8, 10, 10-

octachlorobornane (B8-1413) and 2-endo, 3-exo, 5-endo, 6-exo, 8, 8, 9, 10, 10-nonachlorobornane (B9-1679).

1.4 Bioaccumulation and Trophic Transfer of Organochlorines

Bioaccumulation is the process in which the chemical concentration in an organism achieves a level that exceeds that in the water as a result from all possible exposure and elimination routes (Gobas and Morrison 2000). In fish and other aquatic organisms, bioaccumulation can occur through diet, diffusion across respiratory membranes, and absorption through the skin (Thomann 1989, Mackay and Fraser 2000). Accumulation from dermal absorption is considered negligible in fish (Spacie and Hamelink 1985). Fish gills are designed for maximum diffusion for respiration, which makes them susceptible to uptake of OCs by passive accumulation from water (Spacie and Hamelink 1985). However, exposure through water only, termed bioconcentration, is thought to be a minor for hydrophobic OCs (log k_{ow} >5) when compared to dietary uptake. The exception is algae, which do not feed. Bioaccumulation factors (BAF) can be used to assess risk of OCs in aquatic ecosystems (Gobas and Morrison 2000).

Bioaccumulation can be quantified using bioaccumulation factors (BAF), often used to assess risk of OCs in aquatic ecosystems (Gobas and Morrison 2000). BAFs can be calculated using the following equation:

$$BAF_{\text{lipid weight}} = [OC_{\text{organism}}]_{\text{lipid weight}}/[OC_{\text{water}}]_{\text{dissolved}} \quad (3)$$

Lipid weights are used to account for different lipid content of various organisms.

Hydrophobicity is considered one of the most important physico-chemical properties in predicting an OCs behavior in the environment, and has been used to assess bioaccumulation of OCs in organisms (Thomann 1989). The log octanol-water partition coefficient (log K_{ow}) is measure of an OCs hydrophobicity, and takes into account solubility in water as well as lipids.

The log K_{ow} is the ratio of the chemical concentration in octanol to that in water at equilibrium; octanol is used as a surrogate for lipids. OCs with log K_{ow}s around 7 were found to have the greatest potential for bioaccumulation (Fisk et al. 1998).

Studies have shown that persistent, hydrophic OCs increase in concentration with increasing trophic position (Evans et al. 1991, Bidleman et al. 1993, Fisk et al 2001), a process termed biomagnification. Biomagnification is "the process in which the chemical concentration in an organism achieves a level that exceeds that in the organism's diet, due to dietary accumulation" (Gobas and Morrison 2000). Because uptake rates are similar for most hydrophobic OCs (Drouillard and Norstrom 2000), the elimination rate is what determines whether a OC will biomagnify (Borgå et al. 2004). Chemicals that are easily eliminated by an organism are less likely to biomagnify than those for which elimination is slow. Specifically, biomagnification occurs because the rate of elimination of OCs is much slower than the uptake rate (Borgå et al. 2004). A biomagnification factors (BMF) can be calculated by comparing the concentration in an organism with that in its diet using the equation:

$$BMF_{\text{lipid weight}} = [OC_{\text{organism}}]_{\text{lipid weight}}/[OC_{\text{diet}}]_{\text{lipid weight}} \quad (4)$$

BMFs are usually only calculated for a single prey species rather than a mixture of species.

Another means to assess biomagnification or trophic transfer of OCs in aquatic food webs is the trophic magnification factors (TMF), previously known as food web magnification factors (Fisk et al. 2001). TMFs have been used to explain the average increase in OCs from one trophic level to the next, while incorporating variability across the entire food web (Borgå et al. 2004). TMFs are also particularly insightful when specific trophic interactions (i.e. predator/prey) are not known and/or when the entire food web is considered. TMFs are based on

the relationship between trophic position, determined using $\delta^{15}N$ (see Section 1.2.3.), and the natural log of the lipid-normalized OC concentration using a simple linear regression:

$$\ln [OC] = a + bTL. \quad (5)$$

A TMF can then be calculated using the following equation:

$$TMF = e^{b}. \quad (6)$$

In this case, *b* is determined from the slope of the relationship between the natural log concentration of OC concentrations (ln [OC]) and trophic position (Fisk et al 2001). TMFs serve as a means to compare OCs and among different regions and ecosystems (Borgå et al. 2004), and have been used to provide ecological insight into the feeding behavior of aquatic organisms (Fisk et al. 2002).

1.5 Estuaries of Brunswick, GA, USA

The estuaries in the Brunswick, GA area are highly contaminated with PCBs (as Aroclor 1268) and toxaphene (Kannan et al. 1997, Kannan et al. 1998, Maruya and Lee 1998a, 1998b, Maruya et al. 2000, Maruya et al. 2001) due to long-term, point-source, industrial discharge. Aroclor 1268 was used by LCP chemicals, a chlor-alkali plant, in Brunswick, GA. Several thousand tons of sludge and process waste were disposed of in surface impoundments constructed along the marshes of Purvis Creek between 1968 until closing in 1991, when LCP Chemicals was declared a Superfund site by the USEPA. To date, in the southeast United States, Aroclor 1268 profile has only been found in biota and sediment in the vicinity of Brunswick, GA (Kannan et al. 1997, Kannan et al. 1998, Maruya and Lee 1998a).

Toxaphene was manufactured in Brunswick, GA by Hercules Inc., and industrial discharge into Dupree Creek has led to high contamination of sediments and biota in both Terry

and Dupree Creeks. Vetter and Maruya (2000) found that sediments, from within 1 km of the former toxaphene plant, had levels of 7300 ng/g total toxaphene (ΣTOX), and levels in biota were also extremely high (990 - 20,000 ± 3300 ng/g wwt). Maruya et al. (2001) found ΣTOX levels in biota that ranged from 1.7 ng/g in blue crab to 26,000 ng/g in mullet.

1.6 Rationale, Objectives, and Hypotheses

The high levels of Aroclor 1268 and toxaphene in Terry and Dupree Creeks provide a unique opportunity to study both the trophic transfer of these contaminants and the feeding ecology of fish and other biota in the estuary. The use of stable isotopes to characterize aquatic food webs and quantify trophic transfer of OCs have been limited primarily to Arctic and sub-Arctic marine and freshwater systems. There is no published data on the trophic transfers of OCs in a temperate estuarine food web. The goals of this project are:

1. Use multiple stable isotopic tracers to determine sources of organic matter (^{13}C, ^{34}S) and trophic position of biota (^{15}N) the estuarine food webs in Terry and Dupree Creeks.

***Hypothesis**: $\delta^{13}C$ and $\delta^{34}S$ will separate major producers in the system*

***Hypothesis**: $\delta^{15}N$ will be a good estimator of trophic position.*

2. Use ^{15}N to assess and quantify the trophic transfer of PCBs and toxaphene across trophic levels in an estuarine food web.

***Hypothesis:** PCB and toxaphene concentrations will increase with increasing trophic level for resident species.*

***Hypothesis:** $\delta^{13}C$ and $\delta^{34}S$ will help explain variability in trophic transfer of PCB and toxaphene among transient species.*

CHAPTER 2

CHARACTERIZATION OF A SOUTHEASTERN U.S. ESTUARINE FOOD WEB USING MULTIPLE STABLE ISOTOPES

2.1 INTRODUCTION

Estuaries are critical habitats because they are productive (Mendelssohn and Morris 2000), important for biodiversity (Craig and Crowder 2000, Kneib 2000, Miller et al. 2000), and are under considerable anthropogenic stress (Mitsch 2000). In the southeastern U.S., salt marshes are a primary component of estuaries; in Georgia alone there are over 1500 km^2 of salt marsh (GA DNR 2005b). Estuaries are also inherently complex ecosystems that are subject to a wide variety of physical, chemical, and biological interactions. Salt marshes in the southeastern U.S. are particularly complex because of extreme tides ($\geq$ 3 m). These tides create tidal creeks, which have two directional flow and reach bankful conditions almost every day of the year (Leeks 1979). This tidal flooding and poor drainage causes anoxia and reduces the overall redox potential of the soils (Armstrong 1976). Salt marshes also are known to be among the most productive ecosystems. *Spartina alterniflora*, the dominant plant species in the lower marsh, is thought to contribute greatly to the productivity of these ecosystems (Long and Mason 1983, Mendelson and Morris 2000). Also, many fish species rely on these estuaries for some portion of there life cycle if not entirely, providing refuge from predators as well amble food resources (Deegan et al. 2000, Litvin and Weinstein 2003, Minello et al. 2003).

Elucidating sources and fate of organic matter and fate in estuaries has been a topic of great interest, particularly determining which producers drive the system (Haines 1976, Sherr 1982, Peterson et al. 1986, Peterson and Howarth 1987, Kwak and Zedler 1997, Hsieh et al. 2002, Chanton and Lewis 2002, Goñi et al 2003, Kaldy et al. 2005). In salt marshes, there are a number of primary producers including smooth cordgrass (*Spartina alterniflora*), phytoplankton, benthic algae, and bacteria. *Spartina alterniflora*, has been credited for the high degree of productivity in salt marshes; early estimates of net primary production of *S. alterniflora* has been

as high as 6656 g m^{-2} y^{-1} (Wiegert 1979). Haines (1977, 1979) also argued that algal production within salt marshes had been underestimated in previous studies and can be important source of carbon to the system; with autochthonous algal input estimated to be as high as 770 g m^{-2} y^{-1} (Haines and Dunstan 1975). However, questions remain regarding the relative importance of marine-based algal and *S. alterniflora* production, as well as freshwater inputs, to secondary production in these systems (Kwak and Zedler 1997, MacAvoy et al. 2000, Chanton and Lewis 2002).

Stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) have, in recent years, become powerful tools in assessing flow of energy and trophic structure in estuarine ecosystems (Peterson 1999, Fry 2002, Chanton and Lewis 2002). Analysis of the natural abundance of stable isotopes can often provide insight into the diet and feeding behavior of organisms because they reflect what an organism has consumed. As well, changes in stable isotopic ratios often occur in a predictable manner and are related to ecological processes. By comparing the isotopic signature of consumers to those of the producers in the system, one is often able to distinguish consumer food sources, establish trophic position, and ultimately the flow of energy.

Carbon stable isotopes ($\delta^{13}C$) can reliably separate plants with the C_3 photosynthetic pathway from those with that utilize the C_4 pathway (Peterson and Fry 1987). C_3 plants are typically more depleted in ^{13}C than C_4 plants, consequently giving C_3 plants a more negative $\delta^{13}C$ value. Habitat of primary producers can also influence $\delta^{13}C$ values, for example, benthic algae tend to be more enriched in ^{13}C, hence having a more positive $\delta^{13}C$ value than pelagic algae. This is the result of carbon limitation created by the larger stagnant boundary layer surrounding benthic plants (France 1995), which reduces photosynthetic selectivity for ^{12}C.

Sulfur isotopes ($\delta^{34}S$) have in recent years become another important tracer of organic matter sources in estuarine food webs, especially when C and N are unable to separate producers (Peterson et al 1986, Kwak and Zedler1997, Hsieh et al 2002, Chanton and Lewis 2002). Sulfur isotopes are able to separate pelagic marine producers such as phytoplankton from those that are terrestrial (Connolly 2004). This is especially useful in estuarine ecosystems where there are a wide variety of potential inputs of organic matter from marine and freshwater sources. Both $\delta^{13}C$ and $\delta^{34}S$ are useful in assess organic matter flow to consumers because their isotopic ratios do not vary greatly from diet to consumer. Together, carbon and sulfur isotopes have been shown to distinguish organic matter that originates from marine phytoplankton, upland plants, and *S. alterniflora* in estuarine systems (Peterson and Howarth 1987, Kwak and Zedler 1997, Hsieh et al. 2002, Chanton and Lewis 2002).

Nitrogen isotopes are useful in constructing trophic relationships in aquatic food webs (Vander Zanden et al 1999). $\delta^{15}N$ increases roughly 3-4 ‰ from diet to consumer (Minagawa and Wada 1984, Hobson and Welch 1992, Cabana and Rasmussen 1994), and when compared to an appropriate baseline, often a primary producer or obligated filter feeder (Fisk et al. 2001), provides a means to quantify relative trophic position of consumers (Vander Zanden et al 1999).

The goal of this study was to use stable isotopes of multiple elements ($\delta^{13}C$, $\delta^{34}S$, $\delta^{15}N$) to trace organic matter flow through an southeastern U.S. estuarine food and establish the trophic position of common primary producers, invertebrates, and fishes. Peterson and Howarth (1987) performed a similar study in the salt marshes of Sapelo Island, GA, USA; however, they focused primarily on benthic invertebrates rather than higher predators (i.e. fish).

2.2 METHODS

2.2.1. STUDY SITE

This study was carried out in Terry and Dupree Creeks; two creeks near Brunswick, GA. Terry and Dupree Creeks lie northeast of Brunswick, GA, USA near the Marshes of Glynn (Figure 2.1). Dupree Creek joins with Terry Creek approximately 2.09 km upstream of the Back River, a tributary to St. Simon's Sound. Like most salt marshes in this area, Terry and Dupree Creeks are influenced greatly by tides that usually vary between 2.0 - 2.5 m, but can reach 3m in vertical height. Salinity can also vary from 9 – 30 ppt with tides and freshwater inputs (Blanton et al. 2001).

2.2.2 FIELD SAMPLING

Spartina alterniflora, seston, sediment, invertebrates and fish (Table 2.1) were all collected from the confluence of Terry and Dupree Creeks from on 9/8/03, 9/25/03, and 12/19/03. Temperature and salinity were measured using a thermometer and refractometer at the beginning of sampling on each date. Fish and invertebrates were collected haphazardly using trawls, 7.5 cm x 7.5 cm gill nets, cast nets and hook and line. Trawl duration was approximately 20 min using a 5 m net with 4.8 cm mesh. Bivalves were removed by hand from the substrate, and *Spartina alterniflora* samples were collected from the creek bank. Seston (i.e. suspended particulate matter) samples were collected by horizontal pulls of a 54 μm mesh, 30 cm diameter zooplankton net, pulled for approximately 100 m by boat. Sediment was sampled using a 20 cm x 20 cm Ponar grab sampler at the confluence of Terry and Dupree Creeks. Sediment samples were placed in clean I-Chem glass jars. All samples were placed immediately on ice and kept frozen at the laboratory until further analysis. Fish and invertebrates were weighed to the nearest 0.1 g and total length was measured to the 0.1 cm upon returning to the lab.

2.2.2 Stable Isotope Analysis

Fish were filleted and muscle tissue was removed just anterior of the dorsal fin for stable isotope analysis. For invertebrates and small fish (< 5 cm), whole individuals were used for analysis. Samples were dried for 24-48 hours at 60°C and then finely ground using a bench grinder. To remove lipids, ground samples were agitated in a 1:2 chloroform to methanol solution for 5 min, centrifuged at 3000 rpm for 2 min, and the supernatant was removed, and the process was repeated. Lipid-extracted samples were dried at 60°C, pulverized, and weighed into approximately 1 μg lots for carbon and nitrogen analysis and approximately 5-6 μg lots for sulfur analysis and placed in tin capsules for stable isotope analysis.

Values of $\delta^{13}C$ and $\delta^{15}N$ concentrations were determined using a Thermo Finnigan DeltaPlus mass-spectrometer (Thermo Finnigan, San Jose, CA, USA) at the Institute of Ecology, University of Georgia (Athens, GA, USA). $\delta^{34}S$ analysis was performed at the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University (Flagstaff, AZ, USA) using a Thermo Finnigan DeltaPlus Advantage mass-spectrometer. All stable isotope data are reported using δ-notation. δ-notation is calculated as:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where X is C, N, or S and R is the ratio $^{13}C:^{12}C$, $^{15}N:^{14}N$, or $^{34}S:^{32}S$ for the sample or standard. $R_{standard}$ values are based on PeeDee Belemnite, Diablo Canyon meteorite, and atmospheric N_2 for $\delta^{13}C$, $\delta^{34}S$, and $\delta^{15}N$, respectively. Using δ-notation, isotopic differences are expressed as per mil (‰).

Poplar and bovine performance standards were run after every 10 samples for quality assurance and quality control of $\delta^{13}C$ and $\delta^{15}N$. A bovine standard was also analyzed for $\delta^{34}S$ quality assurance and quality control.

2.2.3 Data Analysis

Trophic position was calculated from $\delta^{15}N$ using the modified equation from Vander Zanden and Rasmussen (1999):

$$TP_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{ff})/3.4 + 2 \quad (2)$$

where $TP_{consumer}$ is the trophic position of the consumer, 3.4 is the isotopic enrichment factor (Minagawa and Wada 1984), and $\delta^{15}N_{ff}$ is the mean (n = 10) $\delta^{15}N$ of oyster (*Crassostrea virginica*) and green mussel (*Geukensia demissa)*. These non-selective filter feeders are assumed to occupy trophic position 2 (Vander Zanden et al. 1999, Fisk et al. 2001).

Dual isotopes plots ($\delta^{13}C$ vs. $\delta^{15}N$, $\delta^{34}S$ vs. $\delta^{15}N$, $\delta^{13}C$ vs. $\delta^{34}S$) were used to describe approximate relationships between consumers and producers in the estuarine food web. These plots rely on the proximity of a consumer's isotopic signature (e.g. $\delta^{13}C$) to that of a producer to infer dependence on that producer, with the assumption that $\delta^{13}C$ and $\delta^{34}S$ are conserved between trophic positions (Vander Zanden and Rasmussen 2001, Connolly et al. 2004).

Simple linear regression was used to determine if significant relationships existed between $\delta^{15}N$ values and $\delta^{13}C$ or $\delta^{34}S$ values. Stable isotope values were also tested for correlation (Pearson's r) and for normality.

2.3 Results

Ambient air temperatures for the sampling dates 9/8/03, 9/25/03, and 12/19/03 were 23.0, 27.0, and 13.5°C, respectively. The water temperatures were 25.5, 28.5, and 15°C, for the same dates. Salinity for the three sampling date were 15, 22, and 29 ppt.

Stable isotope results for the performance standards showed very good precision and accuracy. The $\delta^{13}C$ values for the poplar and bovine standards were -27.5 ± 0.04 ‰ and -22.1 ± 0.001 ‰, respectively. $\delta^{15}N$ values were -2.54 ± 0.1 ‰ for poplar and 7.6 ± 0.04 ‰ for bovine

standard. $\delta^{34}S$ values for the bovine standard were 7.61± 0.12 ‰. All of these values are within acceptable limits for their respective laboratories, and no correction of stable isotope values for samples were required.

The values of $\delta^{13}C$ ranged from a high of -12.8 ± 0.1 ‰ (mean ± SE) in *S. alterniflora* to a low of -22.8 ± 0.9 ‰ in sediment in the estuary (Table 2.2, Figure 2.2), with seston and all consumers falling between these values. $\delta^{34}S$ values for seston (18.8 ± 0.6‰) and *S. alterniflora* (0.9 ± 5.2 ‰) were taken from Peterson and Howarth (1987); results for samples collected for this study are pending. The consistency of stable isotope values for species collected in this study and Peterson and Howarth (1987) support the substitution of these missing values. Values of $\delta^{34}S$ for all consumers fell in between the values for seston and *S. alterniflora* (Figure 2.3).

Nitrogen isotopic ratios ($\delta^{15}N$) of 4.7 ± 0.2 ‰ and 4.8 ± 0.4 ‰, for sediment and *S. alterniflora* respectively, were very similar (Table 2.2, Figure 2.2). $\delta^{15}N$ values for seston were only slightly higher (5.5 ± 1.3 ‰). $\delta^{15}N$ values for consumers (Figure 2.2) ranged from 6.8 ± 0.2 ‰ in snails (*Melampus coffeus*) to 14.7 ± 0.4 ‰ in longnose gar (*Lepisosteus osseus*). The lowest estimated trophic position from $\delta^{15}N$ values was for sediment (0.94 ± 0.06, Table 2.2). Longnose gar had the highest calculated trophic position of 3.86 ± 0.12.

The linear regressions between $\delta^{15}N$ and $\delta^{13}C$ or $\delta^{34}S$ were not significant. $\delta^{13}C$ values were weakly correlated with $\delta^{15}N$ (r = 0.22) and $\delta^{34}S$ (r = -0.24).

2.4. DISCUSSION

The focus of this study was to identify the primary producers that fuel secondary production in a salt marsh estuary in the Southeast. Values of stable isotopes in primary producers and consumers suggest that both seston and *S. alterniflora* contribute greatly to carbon and energy in this system. This is consistent with Peterson and Howarth (1987), who concluded that seston and

S. alterniflora were the major contributors to secondary production in the salt marshes near Sapelo Island, GA, USA. Considering only fish and invertebrates, it appears that 4 trophic positions are present. Kwak and Zedler (1997) estimated four trophic positions in a southern California, (USA) wetland using an enrichment factor of 3.6. Marine mammals, seabirds, and sharks may potentially represent a fifth trophic position in these systems, although such data are lacking for Terry and Dupree Creeks.

2.4.1. Sources of Primary Production

Seston and *S. alterniflora* are driving secondary production in Brunswick estuaries, with evidence that seston may be more important. As expected, because it is a C_4 plant, *S. alterniflora* had the most enriched $\delta^{13}C$ values in the food web (Haines and Montague 1979, Peterson and Howarth 1987, Kwak and Zedler 1997). The $\delta^{13}C$ value for seston was more depleted than that observed in *S. alterniflora*, likely due to its composition of marine-based algae and bacteria, and potentially upland (C_3) plants (estimates of $\delta^{13}C$ in seston include: -22.2 ± 0.4 ‰ Haines and Montague 1976, -22.4 ± 0.05 ‰ Chanton and Lewis 1999, -23.1 ± 0.17 ‰ Currin et al. 2003). However, the $\delta^{34}S$ signature of seston, which is enriched compared to upland plants (Peterson and Howarth 1987), suggests that upland plants are of minor importance in the estuary seston. Values of $\delta^{34}S$ also varied greatly between seston and *S. alterniflora*, which allow their relative importance to secondary consumers to be assessed. Caution is warranted when interpreting the $\delta^{34}S$ values of primary producers, since they are currently substituted from Peterson and Howarth (1987). A comparison of the $\delta^{13}C$ and $\delta^{34}S$ values (Figure 2.4) of the consumers in this study suggests that seston may be more important in supporting secondary production in this system.

Stable isotope values in sediment provide an integrative indicator of carbon input into estuaries. Values of $\delta^{13}C$ in the sediment had high variability and were the most depleted of the

samples analyzed (Figure 2.2). This suggests that input from upland (C_3) plants, which have a lower $\delta^{13}C$, but are similar to results of sediment from Sapelo Island, GA USA (Sherr 1982). The dynamics of the estuary, particularly tidal flux, may cause isotopic values of sediment and seston to vary with season and time of collection (Currin et al. 2003) suggesting upland plants may be a source of carbon for the estuary at different times of the year. Peterson and Howarth (1987) concluded reliance on seston and *S. alterniflora* varied between spring and fall, but upland plants were not major contributors to the system, although they did not assess sediment. The lack of stable isotope data for sediment (pending) limits our ability to assess the importance of seston, upland plants and *S. alterniflora* in the estuary. There may also be many bacteria-driven reactions that may alter carbon isotopic signatures of organic matter in sediments.

The $\delta^{15}N$ values of sediment, *S. alterniflora* and seston were all similar and suggest an expected low trophic position. These values are ~3.3 ‰ lower than those of oysters and mussels, which if these filter feeders are assumed to occupy trophic position of two, is consistent with a trophic position of one for primary producers. This suggests that the use of an enrichment factor of 3.4 is appropriate for this system. Seston was slightly more enriched in ^{15}N than sediment or *S. alterniflora*, which is similar to what was reported for a coastal wetland in California, (USA) (Kwak and Zedler 1997).

2.4.2. Isotopic Ratios in Consumers

A comparison of $\delta^{13}C$ and $\delta^{34}S$ values of consumers in the estuary of this study shows that most lie in a continuum between seston and *S. alterniflora* (Figure 2.4), suggesting that most consumers do not rely exclusively on either of these two producers and that upland plants are not important. These results were very similar to those reported for another Georgia estuary by Peterson and Howarth (1987).

The relative position of consumers on the continuum provides an estimate of how seston and *S. alterniflora* are utilized by consumers; proximity determines reliance. For example, oysters and mussels appear to depend highly on seston as a food source, due to their close proximity in stable isotope values, which is consistent with previous results for filter feeders (Peterson and Howarth 1987, Chanton and Lewis 2002). The more negative $\delta^{13}C$ suggests some input from sediment or upland plants, or that seston values are variable. Note, the current lack of $\delta^{34}S$ data for sediment hinders interpretation. Filter feeders may also be able to integrate changes in carbon and nutrient flow in the estuary over time, and consequently integrate temporal variation in isotopic ratios; they may be useful endpoints for isotopic comparisons. A better understanding of the influence of physiology and the environment on the turnover of stable isotopes in biota would improve our ability to interpret these results.

Although most consumers in the Bruswick estuary lie within seston-*S. alterniflora*, $\delta^{13}C$-$\delta^{34}S$ continuum, based on a comparison of $\delta^{13}C$ and $\delta^{34}S$ values (Figure 2.4), consumer values were closer to seston, at least for the species studied. This implies that seston is a greater contributor of carbon to this system than *S. alterniflora*. Hughes and Sherr (1983) also concluded that algal production contributed more to secondary production than *S. alterniflora* in the salt marshes of Sapelo Island, GA (USA). Early studies have shown that the productivity of *S. alterniflora* from Sapelo Island, GA (USA) could be as high as 6656 g C m^{-2} y^{-1} (Wiegert 1979). More recent studies, however, have estimated the production of *S. alterniflora* to be 1421 g C m^{-2} y^{-1} (Dai and Wiegert 1996). The later is much lower than previously believed, but this estimate is still an order of magnitude higher than estimated phytoplankton production (171 g C m^{-2} y^{-1}, Haines and Dunstan 1975). The greater reliance of consumers on phytoplankton could be due to decreased formation of detritus during the winter months (Teal 1962). Even though production of

S. alterniflora is much higher than that of phytoplankton, detrital inputs may greatly reduced at certain times of the year forcing consumers to utilize autochthonous carbon.

Deposit-feeding invertebrates such as shrimp and snails tended to be more depleted in ^{34}S and enriched in ^{13}C than pelagic or suspension-feeders. This is most likely due to their greater reliance on benthic and/or detrital food source (e.g. *S. alterniflora*) (Deggan and Garritt 1997). Squid (*Lolliguncula brevis*) had high $\delta^{34}S$ values similar to those of suspension feeders, but the $\delta^{13}C$ values were more similar to those of benthic invertebrates. Squid are predatory and represent a higher trophic position based on their $\delta^{15}N$ values (Table 2.2), which could explain why their $\delta^{13}C$ and $\delta^{34}S$ values are more centered between seston and *S. alterniflora* than other invertebrates. Since squid are predators, they may have access to a wider variety of food choices than lower trophic position organisms (e.g. oysters).

Longnose gar (*Lepisosteus osseus*) is a freshwater species that enters brackish waters to feed (Jones et al. 1978). *L. osseus* is picsivorous, as reflected in their high $\delta^{15}N$ value (Table 2.2, Figure 2.2, Figure 2.3). The more positive $\delta^{13}C$ and $\delta^{34}S$ values (Figure 2.4) found in the longnose gar were not consistent with values normally associated with freshwater species (i.e. C3 plants); rather, their isotopic composition is more similar to estuarine dependent species (e.g. *Cynoscion nebulosus*). These isotopes turnover very slowly in fish and are driven in large part by growth (Hesslein et al. 1993). This implies that longnose gar may utilize these estuaries on more of a long term basis (i.e. year-round). On the contrary, American shad (*Alosa sapidissima*, n = 1), had an isotopic signature that was more similar to upland (C_3) plants, i.e. more depleted in ^{13}C and ^{34}S (Peterson and Howarth 1987, Kwak and Zedler 1997). This would suggest an influence of terrestrial organic matter, consistent with the fact that as an anadromous species, typically enter freshwater in the spring (MacAvoy et al. 2000). Based on the length (217 mm) and weight

(0.2 kg) the shad was likely a juvenile, which spend their first summer in freshwater (Facey and Van Den Avyle 1986).

The use of $\delta^{15}N$ values was a good estimator of trophic position (TP) of biota in this estuarine food web based on life history characteristics (Table 2.2, Table 2.3). Based on the $\delta^{15}N$ values, most of the invertebrates occupied a trophic position of approximately 2, with the exceptions being snails (*Melampus coffeus*) and shrimp (*Penaeus setiferus*). The lower TP of snails (1.56 ± 0.07) could be related to a more intimate relationship with the sediment and associated detrital food web (Deegan and Garritt 1997). It could also be an artifact of having sediment in the gut, since the gut was not removed prior to analysis. *P. setiferus* occupied a slightly higher TP than other invertebrates which could be due to occasional scavenging upon decaying fish or invertebrates that had higher $\delta^{15}N$ values (Muncy 1984). Most fish species collected from the estuary had a TP of approximately 3, which implies that these species have a similar diet and feed predominantly on invertebrates and small fish, which is consistent with preferred prey items (Table 2.3). Piscivorous fish species such as longnose gar (*Lepisosteus osseus*), which had the highest calculated TP (3.9 ± 0.12).

Body size appeared to influence $\delta^{15}N$ values for weakfish (*Cynoscion regalis*) and mullet (*Mugil cephalus*) (Table 2.2), the only species where a range of sizes were collected. Smaller fish of both species had lower $\delta^{15}N$ values compared to larger individuals of the same species, suggesting that trophic position increases with body size. Smaller mullet (<15 cm) were statistically significant from larger individuals (t-test, d.f. = 8, $p = 0.002$). Similar comparisons could not be made for weakfish; there was only a single individual in the larger size range. Trophic position of fish is has been shown to be correlated with body size (Johnson et al. 2002). This would suggest that smaller individuals of these species were feeding at a lower trophic

position. In general, there was a positive relationship with body size and $\delta^{15}N$ values ($r^2 = 0.20$, $p < 0.001$).

With increasing $\delta^{15}N$, the $\delta^{13}C$ values of consumers tended to converge on values intermediate of those of *S. alterniflora* and sediment (Figure 2.2). A similar pattern was seen when $\delta^{34}S$ values were compared to $\delta^{15}N$ (Figure 2.3), however most consumer converged between seston and *S. alterniflora*. Fish that occupy higher trophic positions likely have a wide variety of prey items resulting in an integration of carbon sources, whereas lower trophic consumers may be more limited in diet items. For example, seatrout (*Cynoscion nebulosus*) have a wide variety of diet items (Table 2.3) that have different isotopic signatures, and as a result has an isotopic signature that is reflective of incorporating various prey into its diet. Kwak and Zedler (1997) saw a similar relationship with $\delta^{15}N$ and $\delta^{34}S$ in biota collected from a coastal wetland in southern Californina, USA. As well, similar relationships and conclusions between $\delta^{15}N$ and $\delta^{13}C$ in ocean food webs have been observed (Hobson et al. 1998, 2002).

2.5. CONCLUSIONS

Contributions of seston and *S. alterniflora* to secondary production in a coastal salt marsh estuary could be assessed with the use of multiple stable isotopic tracers (^{34}S, ^{15}N, ^{13}C). As shown in previous studies in these ecosystems (Peterson et al. 1986, Peterson and Howarth 1987), both seston and *S. alterniflora* play a major role in supporting higher trophic levels. The majority of consumer species relied upon a mixture of seston and *S. alterniflora*, but it appears that seston may be more important in supporting secondary production in this system. Consumers within the seston-*S. alterniflora* continuum, are shifted more towards seston rather than being evenly dispersed between the two endpoints. It also appears that these tidal creeks are important for longnose gar, a freshwater species, and may rely heavily on these estuaries for

feeding. This is reflected by their isotopic signatures are more similar to estuarine dependent species than that of a fish feeding in freshwater ecosystems. $\delta^{15}N$ was also shown to be a good estimator of trophic position for biota in the estuary. In general, there was good agreement between potential prey items of consumers and trophic position. Body size affected $\delta^{15}N$ values, and consequently trophic position. This was shown across and among species (i.e. mullet).

As in previous studies multiple stable isotopes have the ability to determine sources of primary production and link primary and secondary production in esturaries. Seston and *S. alterniflora* are both important in fueling the secondary production of salt marsh consumers as shown by using stable isotopes of carbon and sulfur. Utilizing stable isotopes of nitrogen has also been shown to be effective at estimating trophic position of biota in the estuary. This study shows that stable isotopes continue to be valuable tools in understanding trophic interactions and structure of estuarine food webs.

Table 2.1: Common and scientific names, number of samples analyzed for stable isotopes, and tissue type of biota collected from October to December 2003 from Terry and Dupree Creeks, Brunswick, GA, USA.

COMMON NAME	SPECIES	(n)	TISSUE[d]
PRODUCERS			
Sediment	-	5	
Seston	-	5	
Smooth cordgrass	*Spartina alterniflora*	3	
INVERTEBRATES			
Fiddler Crab	*Uca spp.*	4	W
Ribbed Mussel	*Geukensia demissa*	5	W
Oyster	*Crassostrea virginica*	5	W
Sea lice	*Uhlorchestia spartinophila*	3	W
Coffee bean snail	*Melampus coffeus*	4	W
Squid	*Lolliguncula brevis*	6	W
White Shrimp	*Penaeus setiferus*	6	M
FISH			
American Shad	*Alosa sapidissima*	1	M
Atlantic Menhaden	*Brevoortia tyrannus*	6	M
Atlantic Stingray	*Dayatis sabina*	3	M
Bay Anchovy	*Anchoa mitchilli*	5	W
Blueback Herring	*Alosa aestivalis*	1	M
Hogchoaker	*Trinectes maculatus*	1	M
Ladyfish	*Elops saurus*	1	M
Longnose Gar	*Lepisosteus osseus*	4	M
Mummichog	*Fundulus heteroclitus*	2	W
Oyster Toadfish	*Opsanus tau*	3	M
Red Drum	*Scianeops ocellatus*	1	M
Silver Perch	*Baidiella chrysoura*	5	M
Southern Flounder	*Paralichthys lethostigma*	5	M
Southern Kingfish	*Menticirrhus americanus*	3	M
Speckled Worm Eel	*Myrophis puntatus*	1	M
Spot	*Leiostomus xanthurus*	1	M
Spotted Seatrout	*Cynoscion nebulosus*	4	M
Star Drum	*Stellifer lanceolatus*	2	W
Striped Mullet (large)[c]	*Mugil cephalus*	7	M
Striped Mullet (small)[a]	*Mugil cephalus*	3	M
Weakfish (large)[c]	*Cynoscion regalis*	1	M
Weakfish (small)[b]	*Cynoscion regalis*	6	M
Yellowfin Mojarra	*Eucinostromus argenteus*	6	W

[a]Fish length < 15 cm
[b]Fish length < 10 cm
[c]Fish length > 19 cm
[d]M indicates that muscle tissue was used, W indicates that whole organism was analyzed.

Table 2.2: Stable carbon ($\delta^{13}C$), sulfur ($\delta^{34}S$), nitrogen ($\delta^{15}N$) isotope values, and estimated trophic position (means ± 1 SE) of biota collected from Terry and Dupree Creeks in Fall 2003. Where sample sizes were 2, the range of values are given in parentheses.

ORGANIC MATTER SOURCES	**$\delta^{13}C$**	**$\delta^{34}S$**	**$\delta^{15}N$**	**Trophic Position[d]**
Sediment	-22.78 ± 0.93	pending	4.70 ± 0.20	0.94 ± 0.06
Seston	-18.58 ± 0.28	pending	5.47 ± 1.28	1.16 ± 0.38
Smooth cordgrass	-12.80 ± 0.11	pending	4.76 ± 0.37	0.95 ± 0.11
INVERTEBRATES				
Fiddler Crab	-18.11 ± 0.54	pending	8.37 ± 0.40	2.01 ± 0.12
Ribbed Mussel	-20.74 ± 0.47	15.59 ± 0.31	7.86 ± 0.19	1.87 ± 0.06
Oyster	-20.62 ± 0.32	17.04 ± 0.74	8.67 ± 0.20	2.11 ± 0.06
Sea Lice	-17.93 ± 0.17	18.68 ± 0.19	9.09 ± 0.32	2.23 ± 0.09
Snail	-16.51 ± 0.17	7.66 ± 0.53	6.82 ± 0.23	1.56 ± 0.07
Squid	-17.09 ± 0.48	16.56 ± 0.27	12.22 ± 0.18	3.15 ± 0.05
White Shrimp	-16.38 ± 0.35	7.32 ± 1.15	9.88 ± 0.28	2.46 ± 0.08
FISH				
American Shad	-20.62	5.10	7.48	1.75
Atlantic	-18.87 ± 0.69	14.54 ± 0.38	10.45 ± 0.38	2.63 ± 0.11
Atlantic Stingray	-15.64 ± 0.61	11.36 ± 0.42	12.37 ± 0.19	3.18 ± 0.06
Bay Anchovy	-17.01 ± 0.10	13.02 ± 0.28	13.03 ± 0.14	3.39 ± 0.04
Blueback Herring	-17.21	pending	12.37	3.19
Hogchoaker	-20.18	pending	10.33	2.59
Ladyfish	-20.63	pending	8.92	2.18
Longnose Gar	-19.07 ± .77	12.60 ± 0.97	14.65 ± 0.41	3.86 ± 0.12
Mummichog	-14.95 (-15.39, -14.50)	11.79 (11.77,	10.20 (9.53, 10.87)	2.55 (2.36, 2.75)
Oyster Toadfish	-18.06 ± 0.13	10.69 ± 0.18	11.14 ± 0.25	2.83 ± 0.07
Red Drum	-14.20	9.44	11.02	2.79
Silver Perch	-17.13 ± 0.35	11.66 ± 0.18	11.74 ± 0.33	3.01 ± 0.10
Southern Flounder	-17.31 ± 0.35	12.56 ± 1.03	11.67 ± 0.58	2.99 ± 0.17
Southern Kingfish	-16.00 ± 0.20	11.16 ± 1.54	12.12 ± 1.42	3.12 ± 0.42
Speckled Worm	-19.23	pending	9.29	2.29
Spot	-19.47	pending	8.91	2.17
Spotted Seatrout	-16.10 ± 0.78	10.58 ± 0.68	12.69 ± 0.51	3.29 ± 0.15
Star Drum	-17.09 (-17.11, -17.06)	12.27 (11.40,	11.09 (10.65,	2.81 (2.69,
Striped Mullet	-17.65 ± 0.44	9.71 ± 0.88	9.16 ± 0.29	2.25 ± 0.09
Striped Mullet	-16.68 ± 1.15	pending	6.94 ± 0.37	1.60 ± 0.11
Weakfish (large)[c]	-18.03	14.19	14.11	3.70
Weakfish (small)[b]	-16.55 ±0.17	pending	11.28 ± 0.13	2.87 ± 0.04
Yellowfin Mojarra	-18.50 ± 0.46	6.56 ± 0.93	10.78 ± 0.28	2.72 ± 0.08

[a]Fish length < 15 cm total length
[b]Fish length < 10 cm total length
[c]Fish length > 19 cm total length
[d]Trophic position was calculated according to $TP_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{ff})/3.4 + 2$; where $\delta^{15}N_{ff}$ is the average $\delta^{15}N_{ff}$ of filter feeders.

Table 2.3. Lifespan, diet, salinity tolerances, and migration patterns of selected fish and invertebrate species collected during the fall of 2003 from Terry and Dupree Creeks.

Species	Lifespan	Diet	Salinity	Migration	Reference
Lolliguncula brevis (Atlantic Brief Squid)	~200 days		8.5-35 ppt		Jackson (2004)
Penaeus setiferus (White Shrimp)	12-17 months	detritus, phytoplankton, zooplankton, decaying material	0-27 ppt	migrate offshore to spawn late fall/early winter and return late winter/early spring	Muncy (1984)
Crassostrea virginica (American Oyster)		phytoplankton, bacteria dinoflagellates, detritus	5-40 ppt	sessile	Burrell (1986)
Uca spp. (Fiddler Crab)	1-1.5 years	diatoms, fungi, nematodes,	20-30 ppt	benthic, inhabit marsh year-round	Grimes et al. (1989)
Anchoa mitchilli (Bay Anchovy)		phytoplankton, zooplankton, detritus	0-80 ppt	inhabit estuaries year-round	Morton (1989)
Brevoortia tyrannus (Atlantic Menhaden)	8-10 years	zooplankton, large phytoplankton, detritus	25-40 ppt	migrate offshore to spawn during winter	Rogers & Van Den Avyle (1989)
Paralichthys lethostigma (Southern Flounder)	3-7 years	anchovy, shrimp, menhaden, sea catfish, croaker, mullet	0-30 ppt	migrate offshore to spawn Oct.-Dec.	Music & Pafford (1984), Reagan and Wingo (1985)
Dayatis sabina (Atlantic Stingray)	~9 years	worms, shrimps, bivalves	0-45 ppt	Inshore shallow from spring thru summer, deep holes during winter, high site fidelity	Snellson et al. (1988)
Opsanus tau (Oyster Toadfish)	6-8 years	crabs, shrimp, fish, squid		Sedentary, non-migratory	Wilson et al. (1982)
Menticirrhus americanus (Southern Kingfish)	3-5 years	shrimp, crabs, worms, fish	<10-20ppt	migrate offshore during winter	Music & Pafford (1984), Sikora & Sikora (1982)
Mugil cephalus (Striped Mullet)	~4 years	Detritus, benthic algae, inorganic sediment	0-35 ppt	migrate offshore to spawn in fall and winter	Collins (1985)
Cynoscion regalis (Weakfish)	6-11 years	clupeids, anchovies, shrimp, fish, squid	0.1-32.3 ppt	adults migrate into estuaries from spring to fall	Mercer (1989), Music & Pafford (1984)
Cynoscion nebulosus (Spotted Seatrout)	8-12 years	shrimp, crabs, pinfish, mullet, anchovies, menhaden	15-35 ppt	inhabit estuaries year-round	Mercer L.P. (1984), Johnson and Seaman (1986)
Alosa sapidissima (American Shad)	5-6 years	copepods, bosmids, daphnids, mysids	0-35 ppt	anadromous, spawn in FW, migrate offshore	Facey and Van Den Avyle (1986)
Lepisosteus osseus (Longnose gar)	~17 years	fish		Freshwater, occasionally entering brackish	Ross (2001)
Fundulus heteroclitus (Mummichog)	4 years	plankton, grass shrimp, polychaetes	0-32 ppt	non-migratory, small home range	Abraham (1985)
Scianops ocellatus (Red Drum)	6-7 years	fish, shrimp, crabs	5-50 ppt	move into deep water to spawn in late summer	Reagan (1985)
Elops saurus (Ladyfish)	6 years	fish, shrimp	0-45 ppt	spawn in open ocean through out the year	Palko (1984), Zale and Merrifield (1989)

Figure 2.1: Sample location for stable isotope of taken from Terry and Dupree Creeks, Brunswick, GA, USA during the fall of 2003. The sample site is indicated by the black dot and arrow.

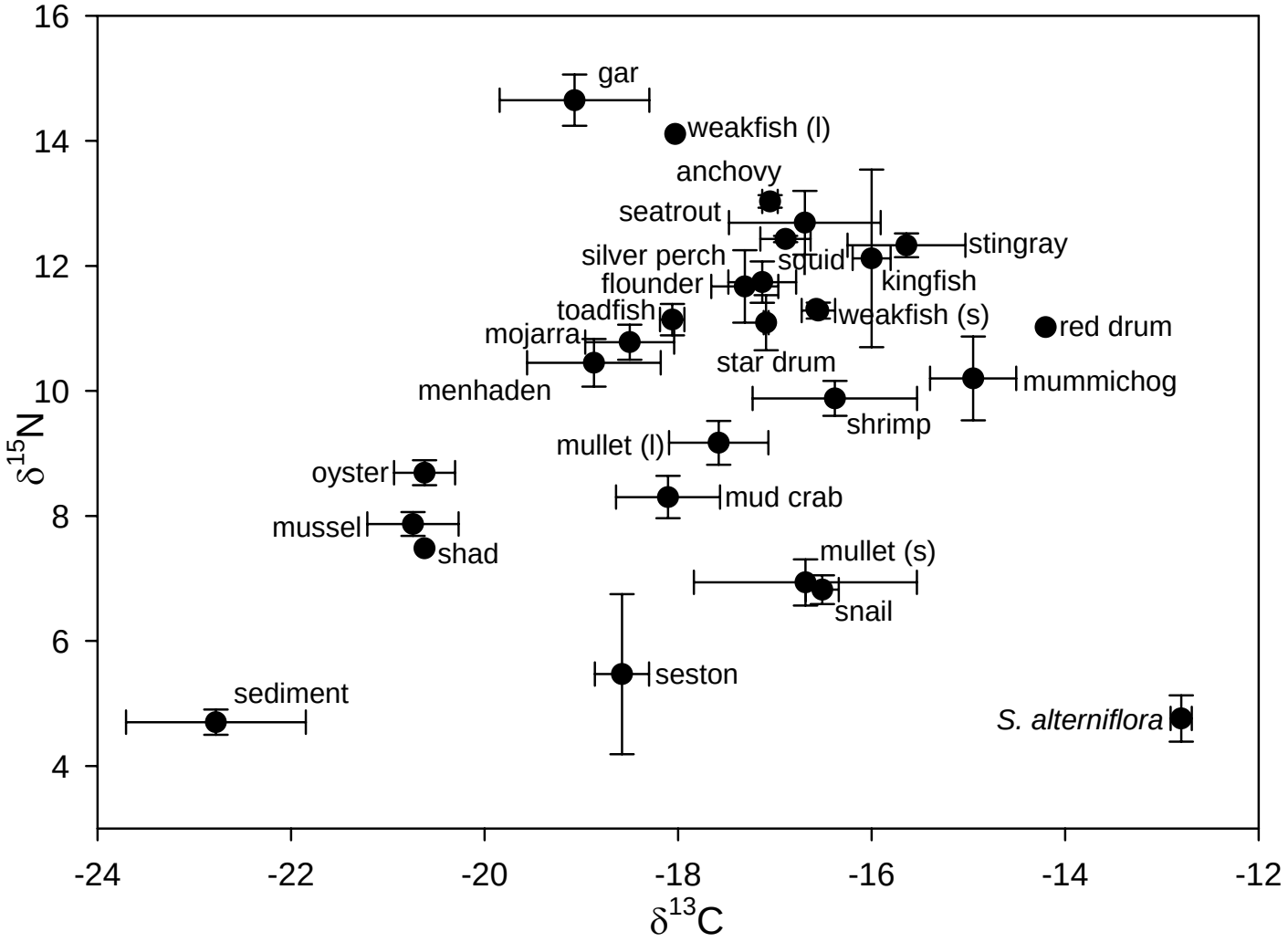

Figure 2.2: Dual isotope plot of $\delta^{13}C$ vs. $\delta^{15}N$ (mean ± SE) of salt marsh producers and consumers from Terry and Dupree Creeks, Brunswick, GA, USA. See Table 2.3 for indication of species' roles.

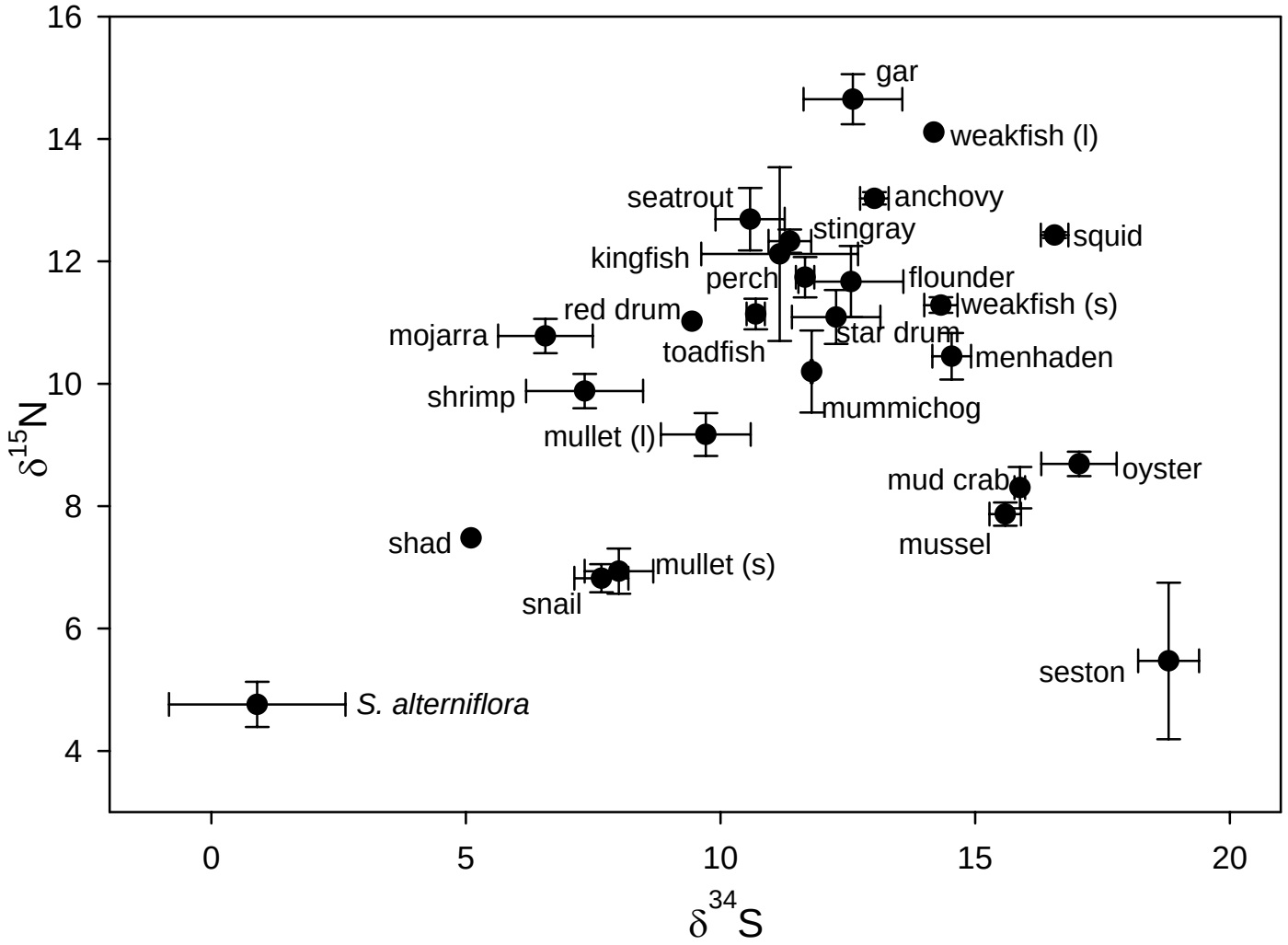

Figure 2.3: Dual isotope plot of $\delta^{34}S$ vs. $\delta^{15}N$ (mean ± SE) of salt marsh producers and consumers from Terry and Dupree Creeks, Brunswick, GA, USA. See Table 2.3 for indication of species' roles. $\delta^{34}S$ data for seston and S. alterniflora are taken from Peterson and Howarth (1987).

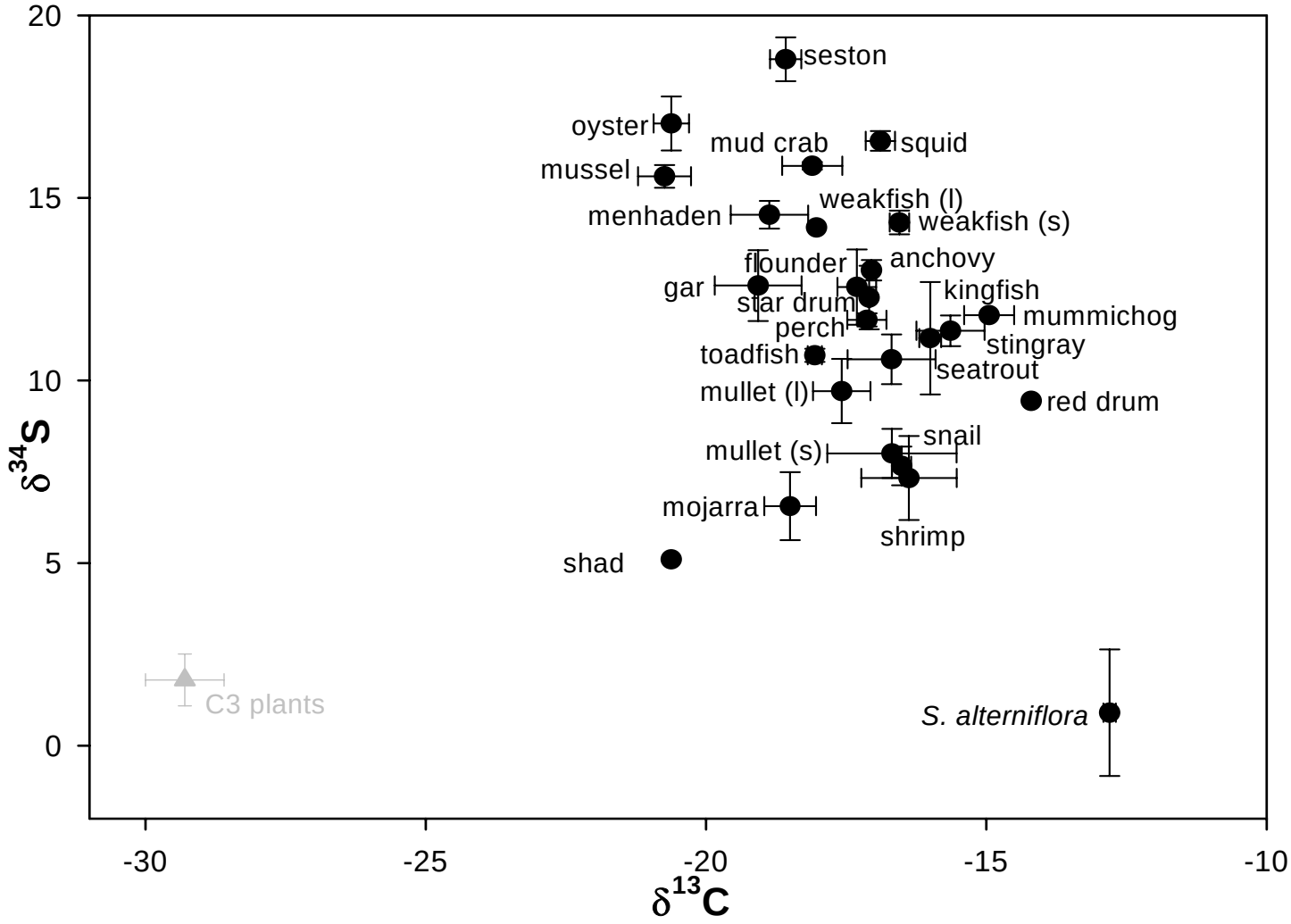

Figure 2.4: Dual isotope plot of $\delta^{13}C$ vs. $\delta^{34}S$ (mean ± SE) of salt marsh producers and consumers from Terry and Dupree Creeks, Brunswick, GA, USA. See Table 2.3 for indication of species' roles. Data for C3 plants were taken from Peterson and Howarth (1987).

CHAPTER 3

TROHPIC TRANSFER OF ORGANOCHLORINES IN A SOUTHEASTERN U.S. SALT-MARSH ESTUARY

3.1 INTRODUCTION

Organochlorines (OCs) include an assortment of anthropogenic contaminants that have been used for industrial and agricultural purposes. OCs are chlorinated hydrocarbons that are characterized by having low solubility in water, an affinity for lipids, and resistance to biodegradation (de March et al. 1998). Many of these compounds are toxic, and along with these physicochemical properties make OCs a concern for human and wildlife health. Although many OCs have been banned or their use restricted in many countries, they are still found at significant concentrations in the environment (Evans et al. 1991, Bidleman et al. 1993, Whittle et al 2000, Fisk et al. 2002, Borgå et al. 2005).

Organochlorines have a tendency to move into and accumulate in aquatic systems, and most of the historical problems associated with these chemicals have been with aquatic organisms. For example, egg shell thinning in bald eagles, which decimated North American populations in the 1960s and 70s (Wiemeyer et al. 1993), was due to high levels of DDT in fish prey (Glaser and Connolly 2002). As well, most states in the U.S. have fish consumption restrictions or guidelines (e.g. GA DNR 2005a) for some species due to unsafe OC concentrations, which are rare for terrestrial animals.

Central to the OC problems in aquatic systems is the potential for bioaccumulation and trophic transfer. Bioaccumulation is the process in which the chemical concentration in an organism achieves a level that exceeds that in the water as a result from all possible exposure (i.e water, diet) and elimination (i.e. water, fecal, biotransformation, growth) routes (Gobas and Morrison 2000). For many hydrophobic OCs (those with a log octanol-water coefficient (k_{ow}) > 4-5), such as PCBs and toxaphene, diet, as opposed to water, is the dominant route of exposure in fish (Thomann et al. 1992). The movement of OCs from one trophic level to the next is termed

trophic transfer (Gobas and Morrison 2000). For recalcitrant and hydrophobic OCs, trophic transfer often results in biomagnification, which is — "*the process in which the chemical concentration in an organism achieves a level that exceeds that in the organism's diet, due to dietary accumulation*" (Gobas and Morrison 2000). Trophic transfer and/or biomagnification can be quantified by calculating a biomagnification factor (BMF) using the equation:

$$BMF_{\text{lipid weight}} = [OC_{\text{organism}}]_{\text{lipid weight}}/[OC_{\text{food}}]_{\text{lipid weight}} \quad (1)$$

This equation assumes the concentration of the OC in the organism, $[OC_{\text{organism}}]$, is in equilibrium, or at least steady state with its food. Lipid-corrected concentrations are used to account for differences in lipid content among and between species. If an OC's BMF is greater than 1 it is considered to biomagnify, although if it is less that 1 trophic transfer can still be a significant process.

Biomagnification factors are highly dependent on the prey used as $[OC_{\text{food}}]$ in equation 1 (Moisey et al. 2001), and can be difficult to determine if the organism has a wide variety of prey species. This can often be reduced by estimating a trophic magnification factor (TMF) (Fisk 2001). TMFs have been used to explain the average increase in OCs from one trophic level to the next and avoid complications associated with choosing prey items by incorporating variability across the entire food web and using a non-specific estimator of trophic position ($\delta^{15}N$) (Borgå et al. 2004, Ruus et al. 2002, Fisk et al. 2001). However, they require data from a range of species in the food web, and rarely provide information if data are only available for a single species (Borgå et al. 2004). TMFs are based on the relationship between trophic position, determined using $\delta^{15}N$ (see Section 1.2.3.), and the natural log of the lipid-normalized OC concentration (Fisk et al. 2001). They are estimated from the equation:

$$TMF = e^{b} \quad (2)$$

where b is the slope derived from the linear regression:

$$\ln [OC] = a + bTP. \quad (3)$$

where, a is a constant, [OC] is the lipid-corrected concentration of the OC in the organism, TP is the organism's trophic position based on $\delta^{15}N$, and b is the slope of the line produced by a simple linear regression between the two terms. TMFs can be used to compare the trophic transfer of OCs across different regions and ecosystems and to identify important factors that influence OC concentrations in aquatic organisms (Borgå et al. 2004). These relationships can also be used for insight into the feeding behavior and ecology of aquatic organisms (Fisk et al. 2002).

Predominantly, the use of TMFs to assess trophic transfer of OCs has been confined to arctic marine (Fisk et al. 2001, Ruus et al. 2002, Hoekstra et al. 2003) and freshwater (Kidd et al. 1995) ecosystems. To date there has been no published data on the trophic transfer of OC in estuarine ecosystems utilizing TMFs. The lack of TMF studies in estuaries may be due in part to the complexity of estuaries, where carbon sources, and likely contaminants, can come from many different sources (i.e. terrestrial, freshwater, and marine) (Peterson et al. 1986, Peterson and Howarth 1987, Chanton and Lewis 2000). As well, many fish and invertebrate species do not utilize estuarine resources for their entire life span, which could blur the ln[OC]-$\delta^{15}N$ relationship because of multiple $\delta^{15}N$ and OC sources. This is of particular issue in southeastern U.S. estuaries, which are highly complex due to extreme tides ($\geq$ 3 m), anoxic soils, high productivity (Long and Mason 1983, Mendelson and Morris 2000), which provides for wide variety of fish and invertebrate species (Deegan et al. 2000, Litvin and Weinstein 2003, Minello et al. 2003).

Terry and Dupree Creek are two tidally influenced creeks located near Brunswick, GA, USA that are highly contaminated with OCs. Long-term unregulated industrial discharge from

former chlor-alkali and toxaphene plants in the vicinity have left sediment and biota severely contaminated with PCBs (as Aroclor 1268) and toxaphene in the salt marshes in this area (Kannan et al. 1997, Kannan et al. 1998, Maruya and Lee 1998a, Maruya and Lee 1998b). Aroclor 1268 is a unique mixture of heavily chlorinated PCB congeners (68% chlorine by mass) which dominate PCB profiles of sediment and biota in the estuary. Toxaphene is a pesticide, composed of approximately 200 chlorinated camphenes and bornanes, which was commonly used in cotton farming in the southern U.S.A. Sources of these two mixtures are unique to the Brunswick area and high levels allow detection of less common components of the technical mixtures, specifically toxaphene, and provide an excellent opportunity to study trophic transfer of these contaminants in an estuary.

Toxaphene and PCBs have been shown to biomagnify based on laboratory (Fisk et al. 1998) and field studies (Evans et al. 1991, Fisk et al. 2001), but their behavior in estuaries is much less understood. Determination of TMFs of OCs in estuaries may be difficult due to the inherent complexity of the ecosystem. Many estuarine fish species migrate offshore to spawn or have wide habitat ranges, which could be an issue when dealing with a point source such as the Brunswick, GA area as opposed to the Arctic where OC contamination is predominantly the product of long-range transport from southern regions with a few point sources (Hoff et al. 1996). This movement away from the point source could allow the organism to depurate and reduce its contaminant load.

Stable isotopes of carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$) have been used in previous studies to distinguish marine from freshwater sources of organic matter, and consequently feeding ecology of fish (MacAvoy et al. 2000, Fry 2002, Chanton and Lewis 2002). This is especially useful in estuarine ecosystems where there are a wide variety of inputs of organic matter from marine and

freshwater sources (Peterson et al. 1986, Peterson and Howarth 1987, Kwak and Zedler 1997, Hsieh et al. 2002, Chanton and Lewis 2002). Both $\delta^{13}C$ and $\delta^{34}S$ are useful in assess organic matter flow to consumers because their isotopic ratios do not vary greatly from diet to consumer. By incorporating $\delta^{13}C$ and $\delta^{34}S$ into the assessment of trophic transfer of OCs, it may help reduce the variability associated with migratory patterns and feeding relationships of estuarine biota.

The aims of this study were to assess trophic transfer, using multiple stable isotopes, and quantify TMFs of PCB and toxaphene congeners in the food web of Terry and Dupree Creek and compare them to those found in arctic systems. In order to assess risks and fate of OCs, it is important to understand how these compounds bioaccumulate in biota and move through estuarine food webs

3.2 Methods

3.2.1. Site Description

Marsh fauna were collected from the confluence of Terry and Dupree Creeks, Brunswick, GA (Figure 3.1). These creeks lie on the northeast side of the Brunswick peninsula. This site was chosen based on its proximity to known point sources of PCBs and toxaphene, from two Superfund sites. A former toxpahene plant (Hercules Inc.) produced technical grade toxaphene and waste effluent was discharged into a ditch that flowed into Dupree Creek. LCP Chemicals was a chlor-alkali plant located west of Brunswick, GA, USA along Purvis Creek. LCP Chemicals disposed of waste consisting of mostly PCBs (as Aroclor 1268) and mercury into surface impoundments built along the marsh. Long-term discharge has left residual contamination of sediment and biota of the salt marshes surrounding Brunswick, GA, USA.

3.2.2 Field Collections

Invertebrates and fish (Table 3.1) were all collected from the confluence of Terry and Dupree Creeks from October to December of 2003. Temperature and salinity were measured on each sample date (See section 2.3). Fish and invertebrates were collected haphazardly using trawls, 7.5 cm x 7.5 cm gill nets, cast nets and hook and line. Trawl duration was approximately 20 min using a 5 m net with 4.8 cm mesh. Bivalves were removed by hand from the substrate. All samples were placed immediately on ice and kept frozen until further analysis. Fish and invertebrates were weighed to the nearest 0.1 g and length was measured to the 0.1 cm.

3.2.3 Organochlorine Analysis

3.2.3.1 Sample Processing

Fish and invertebrates were weighed on a top loading balance to the nearest 0.01 g, and fork length or carapace width was recorded to the nearest 0.1 cm. Fish were filleted using a solvent-rinsed cutting board and knife. For smaller fish and invertebrates composites of whole individuals were used. Sub-samples of tissue were collected for stable isotope analysis. Composites or muscle tissue were homogenized with a commercial blender (Waring Heavy Duty Laboratory Blender, Torrington, CT.). The stainless steel blender carafe was thoroughly detergent washed and rinsed with acetone and hexane between samples.

3.2.3.2 Sample Extraction for Organochlorines

OC extraction methods are those outlined by Pulster et al. (2005). Briefly, approximately 5 g of tissue homogenate was ground with 30 g kiln-fired (~500°C for 13 hours) sodium sulfate (Na_2SO_4) using a solvent-rinsed ceramic mortar and pestle. The homogenized sample mixture was packed into a 33-ml stainless steel pressurized fluid extraction cell. Each sample was spiked with ~50 ng of DBOFB and α-HCH each in hexane as recovery surrogates prior to capping the

sample mixture with additional Na_2SO_4 to minimize head space. The extraction cells were then loaded into an Accelerated Solvent Extractor (ASE Model 200; Dionex, Sunnyvale, CA) and were extracted three times with CH_2Cl_2 at 100°C and 2000 psi. Each ASE cycle consisted of a 5 min equilibration followed by 5 min static time and a 60 sec purge of 60% of the flush volume using ultra-high purity nitrogen (UHP N_2; >99.999%).

3.2.3.3 Determination of Lipids

Percent extractable lipid was determined gravimetrically from ASE extract. The extract was reduced to 10 ml with UHP nitrogen in a heated water bath and 1.0 ml was transferred into a pre-tared 2.0 ml glass GC vial. The GC vial containing the residue/vial combination was re-weighed after complete solvent evaporation (12-24 h). All mass determinations were made to the nearest 0.1 mg using a (Mettler Toledo, AG245) microbalance. Percent lipid was determined on a wet weight basis using the equation:

$$\% \text{ lipid} = (\text{lipid weight} / \text{sample weight}) * \text{dilution factor} * 100 \quad (4)$$

After lipid determination, the residue was re-dissolved in 1 ml CH_2Cl_2 and was re-combined with the original sample extract. The extract was then reduced and exchanged to hexane and further reduced to 1 ml.

3.2.3.4 Sample Fractionation and Clean up

Florisil column chromatography was used to remove interferences associated with lipids and to separate PCBs from toxpahene. A glass column (500 mm L x 11 mm i.d.) with a fixed 250 ml solvent reservoir was rinsed with acetone and hexane. Kiln-fired glass wool and a stopcock assembly were placed in the bottom of the column and the entire assembly rinsed with copious amounts of acetone followed by hexane. Eighteen grams (±.02 g) of 1.0% deactivated Florisil (60-100 mesh; Fisher Scientific, Fair Lawn, NJ) was dry packed firmly into the column; Na_2SO_4

(stored at 133°C) was added to the top of the Florisil and the sorbent material immediately pre-eluted with 100 ml of hexane. After rinsing, the 1 ml ASE extract was added to the column and eluted first with 90-110 ml of hexane at a rate of 1 drop/sec. A spike test was performed for every Florisil batch to determine the first fraction (F1) volume based on separation efficiency of PCBs and toxaphene. The F1 was reduced to ~1 ml using the TurboVap II (50°C; 12 psi; Zymark, Hopkington, MA) and transferred to a solvent-rinsed 2 ml amber glass GC vial sealed with a Teflon-lined silicone rubber septum. Toxaphene and the slightly more polar pesticides were subsequently eluted at 1 drop/sec with 150 ml of 1:5 CH_2Cl_2:hexane (F2). F2 extracts were reduced to ~5 ml in the TurboVap II, exchanged to hexane, further reduced to $\leq$1 ml, and transferred to a GC vial. Extracts were stored at 20°C until analysis. All solvents used (acetone, hexane and methylene chloride) were of Optima Grade purchased from Fisher Scientific (Fairlawn, NJ).

3.2.3.5 Quantification of Organoclorines

Prior to GC analysis, the volume of each sample extract was adjusted to 1.0 ml by drop wise addition of clean hexane and/or gentle evaporation using UHP nitrogen. Extracts (1 μL injection volume) were analyzed on a Varian 3400CX gas chromatograph with electron capture detection (GC-ECD) and an 8200 48-vial autosampler. Analyte separations were achieved using a 30-m DB-XLB (0.25-mm i.d. x 0.25-μm film thickness; J&W Scientific/Agilent Technologies, Folsom, CA) fused silica column and helium as the carrier gas. The split/splitless injector and detector temperatures were 250°C and 300°C, respectively. The initial oven temperature was 120°C (1 min hold), followed by an increase to 200°C at 10°C/min (1 min hold) and a final increase to 280°C at 2°C/min (10 min hold). Total GC run time was 60 min.

3.2.3.6. CONFIRMATION OF ANALYTES

To confirm the identity of target analytes, one sample per species was analyzed using a Hewlett Packard 6890 Plus Series gas chromatograph coupled to a 5973 mass selective detector (GC-MSD) operating in the negative chemical ionization (NCI) mode. UHP methane at a cavity pressure of ~1.0 x 10^{-4} torr was used as the moderating gas. One microliter (1-µL) was injected into a 30-m DB-XLB (J&W Scientific/Agilent Technologies, Folsom, CA) fused silica capillary column (0.25-mm x 0.25-µm film thickness). Helium was used as the carrier gas with a flow rate of 30 cm/s. The GC oven was programmed as follows: 60°C (1 min hold); increase to 150°C at 10°C/min; increase to 300°C at 4°C/min (11.5 min hold). Total GC run time was 60 min per sample. All sample extracts were analyzed either in the scan or the selected ion monitoring (SIM) mode. Ions monitored and the time windows for SIM analyses for PCBs and toxaphene are shown in Table 3.2.

3.2.3.7 QUALITY ASSURANCE

Analytic accuracy and precision was assured by implementing a performance-based QA/QC program. The program followed standard operating procedures consistent with ultra-trace organics analyses as well as the analysis of procedural blanks, matrix spikes, and Standard Reference Materials (SRMs). Procedural blanks were prepared, processed and analyzed with each analytical batch of field samples to assess the purity of reagents and glassware, as well as to monitor the possibility of laboratory contamination. Procedural blanks underwent the same extraction, fractionation and analytical methods as the field samples. Matrix spikes were performed on a reference field sample, *Cynoscion nebulosus*, collected from Wasaw Sound. Prior to extraction, a field sample duplicate was spiked with known amounts of target contaminants (1.0-ml PCB 2262 99ng/ml). A second set of matrix spikes consisted of spiking the

field sample with known concentrations of particular toxaphene congeners (20-ul TM1 1000ng/ml; 20-ul TM3 1000 ng/ml). The background-corrected recovery of spiked contaminants in the field samples demonstrates the extraction efficiency, providing a measure of analytical precision. SRM-1588a, a cod liver oil SRM (National Institute of Standards and Technology, USA), was prepared, processed and analyzed, providing a measure of precision and accuracy. Measured concentrations were compared with certified values provided by the vendor (NIST, USA). All glassware was borosilicate and thoroughly cleaned between samples. Glassware was detergent washed, kiln-fired and solvent (acetone and hexane) rinsed prior to use. Solvents chosen were based on the polarity of the analytes of interest. All solvents used (acetone, hexane and methylene chloride) were of Optima Grade purchased from Fisher Scientific (Fairlawn, NJ).

3.2.4 Stable Isotope Analysis

Muscle tissue from fish was removed from fish just anterior of the dorsal fin. For invertebrates and fish too small to remove muscle tissue, whole individuals were used for analysis (Table 2.1). Subsamples were taken from *S. alterniflora* and sediment. Samples were dried for 24-48 hours at 60°C and were finely ground using a bench grinder. To remove lipids, ground samples were agitated in a 1:2 chloroform to methanol solution for 5 min, centrifuged at 3000 rpm for 2 min, and the supernatant was removed. This was done twice. Samples were dried at 60°C, pulverized, and divided into approximately 1 μg lots for stable isotope analysis. $\delta^{13}C$ and $\delta^{15}N$ concentrations were determined using a Thermo Finnigan DeltaPlus mass-spectrometer (Thermo Finnigan, San Jose, CA, USA) at the Institute of Ecology, University of Georgia (Athens, GA, USA). $\delta^{34}S$ analysis was performed at the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University (Flagstaff, AZ, USA) using a Thermo Finnigan DeltaPlus Advantage mass-spectrometer.

All stable isotope data are reported using δ-notation. δ-notation is defined as ten times the difference the percent difference in relative isotopic ratios of a sample compared to a standard and calculated as

$$\delta = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (5)$$

where R is the ratio $^{13}C:^{12}C$, $^{15}N:^{14}N$, or $^{34}S:^{32}S$ for the sample or standard. $R_{standard}$ values are based on PeeDee Belemnite, Diablo Canyon meteorite, and atmospheric N_2 for $\delta^{13}C$, $\delta^{34}S$, and $\delta^{15}N$, respectively Using δ-notation, isotopic differences are expressed as per mil (‰). A poplar and bovine standard were ran every 10 samples for quality assurance and quality control.

3.3 Data Analysis

Trophic level was calculated using a method developed Vander Zanden and Rasmussen (1999), which uses δ15N to determine trophic position using the following equation:

$$TP_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{ff})/3.4 + 2 \quad (6)$$

Here, $TP_{consumer}$ is the trophic level of the consumer, and 3.4 is the isotopic enrichment factor. $\delta^{15}N_{ff}$ is the average $\delta^{15}N$ of oyster (*Crassostrea virginica*) and mussel (*Geukensia demissa*), and is used in the equation with the assumption that these organisms occupy trophic position 2 (i.e. non-selective filter feeder). Assuming that filter feeders occupy trophic position 2 has been used in previous food web (Vander Zanden et al. 1999) and TMF (Fisk et al. 2001) studies. TMFs were based on the relationship between trophic position, determined using $\delta^{15}N$, and the natural log of the lipid-normalized OC concentration using a simple linear regression (See Section 3.1, Equation 3).

Based on a review of the literature, only mussels (*Geukensia dimissa*), oysters (*Crassostrea virginica*), anchovies (*Anchoa mitchilli*), silver perch (*Bairdiella chrysoura*), toadfish (*Opsanus tau*), and seatrout (*Cynoscion nebulosus*) were deemed resident species (Table

3.2). Life history characteristics were used to assess migratory patterns and estimated use of estuaries to differentiate resident from transient species (Table 3.2). Resident species are defined as those that are estuarine dependent and year round inhabitants. Transient species are those that have annual migrations offshore or wide range. TMFs calculated for residents included only these species in the regression analysis (Equation 3). All other species collected for this study were classified as transient.

To determine if $\delta^{34}S$ and $\delta^{13}C$ had an influence on organochlorine concentration the following potential multiple regression were used:

$$\ln[OC] = TP + \delta^{13}C + \delta^{34}S + \delta^{13}C * \delta^{34}S + \varepsilon \quad (7)$$

$$\ln[OC] = TP + \delta^{13}C + \delta^{34}S + \varepsilon \quad (8)$$

$$\ln[OC] = TP + \delta^{13}C + \varepsilon \quad (9)$$

$$\ln[OC] = TP + \delta^{34}S + \varepsilon \quad (10)$$

$$\ln[OC] = TP + \delta^{13}C * \delta^{34}S + \varepsilon \quad (11)$$

To determine if all assumptions for multiple regression were met, independent variables were tested for normality and whether they were correlated.

If a PCB or toxaphene congener was not detected in 90% of the samples then they were not regressed against trophic position. For congeners that did meet the 90% criterion, but still had non-detects for some samples, then one-half the detection limit was used. The use of one-half the detection limit has been used in previous studies for inclusion in statistical analyses (Borgå et al. 2005)

3.4 RESULTS

3.4.1 QUALITY ASSURANCE

The percent recoveries of the two PCB matrix spikes were 83.2 ± 5.3 (mean ± SE) and 88.5 ± 5.2. Seatrout tissue that was spiked with the toxaphene congener mixtures T1 and T3 were 112.4 ± 3.5 and 98.0 ± 1.7, respectively. These recoveries are well within the range considered acceptable for OC analysis.

Stable isotope results for the performance standards showed very good precision and accuracy. The $\delta^{13}C$ values for the poplar and bovine standards were -27.5 ± 0.04 ‰ and -22.1 ± 0.001 ‰, respectively. $\delta^{15}N$ values were -2.54 ± 0.1 ‰ for poplar and 7.6 ± 0.04 ‰ for bovine standard. $\delta^{34}S$ values for the bovine standard were 7.61± 0.12 ‰. All of these values are within acceptable limits for their respective laboratories, and no correction of stable isotope values for samples were required.

All independent variables used in the multiple regressions (trophic position, $\delta^{13}C$, $\delta^{34}S$) were found to be normally distributed. $\delta^{13}C$ was found to be correlated with both trophic position and $\delta^{34}S$. Consequently, only Equation 10 was used to determine if $\delta^{34}S$ could explain variability of in trophic transfer of OCs in the Brunswick estuaries.

3.4.2 STABLE ISOTOPES

The values of $\delta^{13}C$ values ranged from a high of -12.8 ± 0.1 ‰ (mean ± SE) in *S. alterniflora* to a low of -22.8 ± 0.9 ‰ in sediment in the estuary (Table 2.2, Figure 2.2), with seston and all consumers falling between these values. Values of $\delta^{34}S$ for all consumers fell in between the values for seston (18.8 ±0.6 ‰) and *S. alterniflora*(0.9 ± 1.7 ‰) (Table 2.2, Figure 2.3).

Nitrogen isotopic ratios for sediment and *S. alterniflora* were very similar with $\delta^{15}N$ values of 4.7 ± 0.2 ‰ and 4.8 ± 0.4 ‰, respectively (Table 2.2, Figure 2.2). $\delta^{15}N$ values for

seston were only slightly higher (5.5 ± 1.3 ‰). $\delta^{15}N$ value for consumers (Figure 2.2) ranged from 6.8 ± 0.2 ‰ in snails (*Melampus coffeus*) to 14.7 ± 0.4 ‰ in longnose gar (*Lepisosteus osseus*), and suggest a food web with approximately 4 trophic levels. A more thorough discussion of the stable isotope results is found in Sections 2.3 and 2.4.

3.4.3 Organochlorine Concentrations and Profiles

Individual toxaphene and PCB congener concentrations and lipid content for species are summarized in Tables 3.3 and 3.4, respectively. In general, the most common PCB congeners were those associated with Aroclor 1268, which included congeners 187, 202, 199, 196, 208, 206. Exceptions to this were mussels and shrimp that had higher abundances of lower chlorinated PCBs such as 153 and 105. The dominant toxaphene congeners in all biota were B6-923 (Hx-Sed) and B7-1001 (Hp-Sed). Other common congeners included B8-1945 (P-41), B8-1414 (P-40), B8-806/B8-809 (P-42a/b), B8-1413 (P-26), and B9-1679 (P-50).

3.4.4 Trophic Magnification Factors

For most PCB congeners, there was no significant relationship between the lipid-corrected OC concentration and trophic position for most congeners. Only PCB 118, -153, -187, -202, -180, -199, -196, -194, -206, and -209 met the 90% criterion for regression analysis. Of the congeners regressed against trophic position, only PCB 187 ($r^2 = 0.13$, p=0.03), -202 ($r^2 = 0.14$, p = 0.03), -180 ($r^2 = 0.26$, p = 0.002), and -199 ($r^2 = 0.25$, p = 0.002, Figure 3.2) showed a significant relationship with trophic position. The TMFs calculated for PCB 187, -202, -180, and –199 were 1.87, 2.38, 2.47, and 3.61, respectively (Figure 3.2). When only resident species were used in the regression only PCBs 199 ($r^2 = 0.71$, $p < 0.001$) and 206 ($r^2 = 0.44$, p = 0.014) had a significant relationship with trophic position, however, variability in the associated with the regression was

less than when all species were included in the regression. The TMFs calculated for resident species were 9.54 and 3.92 for PCBs 199 and 206, respectively.

Most toxaphene congeners did not meet the 90% detection criterion established for regression analysis, and the only toxaphene congeners that were compared to trophic position were B6-923 (Hx-Sed), B7-1001 (Hp-Sed), and P-15. There was no significant relationship between toxaphene congener concentration and trophic position when all species were included or when only resident species were used in the determination of TMFs. Hence, no TMFs could be calculated for toxaphene congeners.

3.4.5. Regression with Multiple Stable Isotopes

Multiple regression was used to determine if stable isotopes sulfur could help explain variability associated with trophic transfer of PCBs and toxaphene in the Terry and Dupree Creek food web. When $\delta^{34}S$ was considered in the regression with trophic position, there was no change in which PCB congeners had a significant relationship with trophic position.

None of the three toxaphene congeners assessed (B6-923, B7-1000, and P-15) had a significant relationship when regressed against $\delta^{13}C$. There was a significant relationship found between and $\delta^{34}S$ and B7-1001($r^2 = 0.21$, $p = 0.006$, Figure 3.3) and P-15 ($r^2 = 0.17$, $p = 0.047$). B6-923, B7-1001 and P-15 had no significant relationship in any of the multiple regression that incorporated $\delta^{34}S$. There were no significant relationships between toxaphene congeners and trophic position for resident species.

3.5 Discussion

3.5.1 PCB and Toxaphene Congener Profiles and Concentrations

PCB congener profiles of the biota from Terry and Dupree Creeks were dominated by higher chlorinated (> 7) PCB congeners associated with Arochlor 1268 (Figures 3.4, 3.5),consistent

with those reported from previous studies in this area (Kannan et al. 1997, Kannan et al. 1998, Maruya and Lee 1998a, Maruya and Lee 1998b, Maruya et al. 2001). In most ecosystems, the dominance of such superhydrophobic PCB congeners are rare, and PCB congeners 153 and -138 have the greatest abundance (Fisk and Johnston 1998, Ruus et al. 2002, Hop et al. 2002).

An exception to the high prevalence of superhydrophobic congeners were found in some invertebrate species, which had higher abundances of more ubiquitous, lower-chlorinated PCBs. Bivalve and shrimp had higher amounts of PCBs 118, 105, and 153. Since these bivalves are immobile, their lower levels of higher chlorinated congeners could be due to reduced exposure due to limited transport of Aroclor 1268 congeners from the source to the collection site. Other species collected in this study have the ability to move and feed, or prey upon organisms that feed in the adjoining estuary where Aroclor 1268 concentrations are higher. Shrimp, however are mobile, but migrate offshore at during late fall/early winter (Muncy 1984) as compared to other estuaries, which would affect their PCB congener profile. The results suggest shrimp are likely exposed to higher levels of ubiquitous PCBs along their migration route.

Toxaphene congener profiles were also similar to what have been reported in previous studies (Maruya and Lee 1998a, Maruya et al. 2001). B6-923 (Hx-Sed) and B7-1001 (Hp-Sed) were the most abundant toxaphene congeners in all biota from the study site. Neither of these congeners are major components of the technical mixture and are thought to be dead-end metabolites of reductive dechlorination of the technical mixture in anoxic sediments (Stern et al. 1996, Ruppe et al. 2003). Typically, B6-923 and B7-1001 are not major congeners found in the environment (de Geus et al. 1999). However, at sites that were treated or highly contaminated with toxaphene, and under anoxic conditions, particularly in sediments, they become prevalent in

biota (Miskimmin et al. 1995, Stern et al. 1996, Donald et al. 1998, Maruya and Lee 1998a, Maruya et al. 2001).

Concentrations of PCBs and toxaphene varied greatly among and between species. The highest ΣPCB were found in spotted seatrout (*Cynoscion nebulosus)* (7.0±4.7 μg/g lipid), whereas mussel had the lowest PCB concentration of 0.72 μg/g lipid, respectively. These concentrations are much lower than previously reported by Maruya and Lee (1998b) and Pulster et al. (2005), but collections for those studies were in closer proximity to the source of PCBs than in this study. A single weakfish had the highest ΣTOX_{23} concentrations (31 μg/g lipid). The lowest ΣTOX_{23} were found in blue crab (0.71 μg/g lipid). The high concentrations in weakfish can be accounted for by their higher trophic position, and is similar to what was found in Pulster et al. (2005) for several higher trophic position fish species.

3.5.2 Trophic Magnification Factors

The TMFs calculated for PCBs in this study are similar to BMFs reported for laboratory studies. Fisk et al. (1998) and Buckman et al. (2004) calculated BMFs for several PCB congeners from controlled feeding studies, and found that congeners with seven or more chlorines generally fell in the range of 1-7. These BMFs can be compared to TMFs, because they were controlled lab studies and a TMF is the average increase in contaminant concentration per trophic level. TMFs > 1 also imply that the compound increases with trophic position, and will biomagnify.

All the PCB congeners that had a significant relationship with trophic position had seven or more chlorines and a log $k_{ow} > 7$ (Hawker and Connel 1988). In general, OCs with a log kow ~ 7 are believed to have the greatest potential for bioaccumulation and biomagnification and that congeners with a log $k_{ow} > 7$ biomagnify to a lesser extent (Fisk et al. 1998, Fisk et al. 2001) In fact, many of the PCB congeners (PCB 118 and -153) that have been shown to biomagnify in

food webs did not in this warm temperate estuary. Hop et al. (2002) calculated TMFs for PCBs 118 and -153 in a Barents Sea food web as 3.9 and 4.1, respectively. The TMF for PCB 153 was determined to be 4.4 for an arctic freshwater food web (Kidd et al. 1998). Another study calculated a TMF for PCB 153 to be 6.7 (Fisk et al. 2001), for an arctic marine food web, when marine mammals and seabirds were excluded from the regression. This would suggest that biomagnification processes in warm temperate estuarine systems differ from colder climates and different ecosystems (estuary versus marine or freshwater).

There is very little TMF data for superhydrophobic PCBs, since they are not prevelant in other systems and difficult to quantify. Fisk et al. (2001) determined that PCBs 180 and -187 had TMFs of 10.7 and 7.3, respectively, in an arctic marine food web, which are much higher than what was determined for the same congeners (TMFs for PCB 180 and -187 are 2.47 and 1.87, respectively) from this study. This is likely due to inclusion of marine mammals or seabirds; homeotherms have been shown to increase TMF values (Hop et al. 2002). There was also much higher variability of congener concentration among species from this study, which could account for low TMFs and weak relationships (low r^2) between trophic position and contaminant concentration.

3.5.3 Problems With Assessing Trophic Transfer of Organochlorines in Estuaries

In most instances, there was not a significant relationship between trophic position and PCB and toxaphene congener concentrations in biota collected from Terry and Dupree Creeks. There are a number of factors that could account for this. One explanation is movement of biota in and out of the estuary. Many of the species included in the study are migratory or have wide home ranges (Table 3.2). Organisms that feed in this contaminated estuary for portions of the year may move to locations where concentrations in water and sediment are lower, and thus their exposure is

less. Feeding upon less contaminated diet items in other ecosystems may allow the organism to depurate contaminants and reduced concentrations, which may obscure the [OC]-TP relationship found in the ecosystems of this study. Fisk et al. (2001) found that migratory patterns and feeding habits of seabird affected their contaminant loads, and consequently fell off the [OC]-TP relationship for the ecosystem in which they were collected. In this case, scavenging birds migrated to highly contaminated regions, which greatly increased their contaminant load.

A second explanation may be problems associated with using $\delta^{15}N$ as an indicator of trophic position, in much the same way as described for OCs above. Migrating species may be exposed to food that has varying $\delta^{15}N$ values than those in Terry and Dupree Creeks. Guo et al. (2003) found that the carbon and nitrogen isotopic composition of dissolved organic matter varied within two estuaries. Fish of the same species have also been shown to have different $\delta^{15}N$ values from different lakes (Vander Zanden et al. 1999, Vander Zanden and Rasmussen 2001), which affected their assigned trophic position (Vander Zanden et al. 1999).

Most food web contaminant studies have been confined to the Arctic, and those conducted in warm temperate climates are limited. Environmental factors such as temperature may influence biomagnification of PCBs and toxaphene in marsh biota. For ectothermic organisms such as fish and invertebrates, ambient temperatures influence metabolism; higher temperatures increase metabolic activity. Increased metabolism could, in turn, increase the rate of biotransformation for PCBs and toxaphene congeners that are more biologically active. A lack of relationship between trophic position and biologically active OCs could be the result of warmer temperatures and increased respiration in biota and consequently metabolism of these compounds. Increased metabolism may also account for the differences in biomagnification of the same compounds from different regions (i.e. the Arctic).

3.5.4 Multiple Stable Isotopes and Trophic Transfer of Organochlorines

Most food web contaminant studies that have incorporated stable isotopes other than $\delta^{15}N$ have used only $\delta^{13}C$. Kidd et al. (2001) showed that biomagnification of ΣDDT was greater in pelagic than benthic food webs, as based on $\delta^{13}C$ values of biota. In general, $\delta^{13}C$ and $\delta^{34}S$ did not help reduce variability in the trophic transfer in PCBs and toxaphene in the estuary. The exception was for the toxaphene congener B7-1001 (Figure 3.3). A significant relationship was found between the congeners B7-1001 and P-15 and $\delta^{34}S$. This would suggest that species that rely more on seston as a food source tend to have higher concentrations of this congener.

Table 3.1. Fish and invertebrates collected for organochlorine analysis, number of samples (*n*), and if samples were individuals or composite.

COMMON NAME	SCIENTIFIC NAME	(n)	Individ./Comp.
INVERTEBRATES			
Blue Crab	*Callinectes sapidus*	2	C
Ribbed Mussel	*Geukensia demissa*	2	C
Oyster	*Crassostrea virginica*	1	C
Squid	*Lolliguncula brevis*	2	C
White Shrimp	*Penaeus setiferus*	3	C
FISH			
Atlantic Menhaden	*Brevoortia tyrannus*	5	I
Atlantic Stingray	*Dayatis Sabina*	1	I
Bay Anchovy	*Anchoa mitchilli*	4	C
Oyster Toadfish	*Opsanus tau*	1	I
Silver Perch	*Bairdiella chrysoura*	2	C
Southern Flounder	*Paralichthys lethostigma*	4	I
Southern Kingfish	*Menticirrhus americanus*	2	I
Spotted Seatrout	*Cynoscion nebulosus*	3	I
Striped Mullet	*Mugil cephalus*	4	I
Weakfish	*Cynoscion regalis*	1	I
Yellowfin Mojarra	*Eucinostromus argenteus*	1	C

Table 3.2. Lifespan, diet, salinity tolerances and migration patterns of fish and invertebrate species collected during the fall of 2003 from Terry and Dupree Creek.

Species	Lifespan	Diet	Salinity	Migration	Reference
Lolliguncula brevis (Atlantic Brief Squid)	~200 days		8.5-35 ppt		Jackson (2004)
Penaeus setiferus (White Shrimp)	12-17 months	detritus, phytoplankton, zooplankton,	0-27 ppt	migrate offshore to spawn late fall/early winter and return late winter/early spring	Muncy (1984)
Crassostrea virginica (American Oyster)		phytoplankton, bacteria dinoflagellates, detritus	5-40 ppt	sessile	Burrell (1986)
Geukensia demissa (Green Mussel)				sessile	
Anchoa mitchilli (Bay Anchovy)		phytoplankton, zooplankton, detritus	0-80 ppt	inhabit estuaries year-round	Morton (1989)
Brevoortia tyrannus (Atlantic Menhaden)	8-10 years	zooplankton, large phytoplankton, detritus	25-40 ppt	migrate offshore to spawn during winter	Rogers & Van Den Avyle (1989)
Paralichthys lethostigma (Southern Flounder)	3-7 years	anchovy, shrimp, menhaden, sea catfish, croaker, mullet	0-30 ppt	migrate offshore to spawn Oct.-Dec.	Music & Pafford (1984)
Bairdiella chrysoura (Silver Perch)					
Eucinostromus argenteus (Yellowfin Mojarra)					
Dayatis sabina (Atlantic Stingray)	~9 years	worms, shrimps, bivalves	0-45 ppt	Inshore shallow from spring thru summer, deep holes during winter, high site fidelity	Snellson et al. (1988)
Opsanus tau (Oyster Toadfish)		crabs, shrimp, fish		sedentary	
Menticirrhus americanus (Southern Kingfish)	3-5 years	shrimp, crabs, worms, fish	<10-20ppt	migrate offshore during winter	Music & Pafford (1984), Sikora & Sikora (1982)
Mugil cephalus (Striped Mullet)	~4 years	Detritus, benthic algae, inorganic sediment	0-35 ppt	migrate offshore to spawn in fall and winter	Collins (1985)
Cynoscion regalis (Weakfish)	6-11 years	clupeids, anchovies, shrimp, fish, squid	6.6-32.3 ppt (adults) 0.1-31.7 ppt (juveniles)	adults migrate into estuaries from spring to fall	Mercer (1989), Music & Pafford (1984)
Cynoscion nebulosus (Spotted Seatrout)	8-12 years	shrimp, crabs, pinfish, mullet, anchovies, menhaden	15-35 ppt	inhabit estuaries year-round	Mercer L.P. (1984), Johnson and Seaman (1986)
Callinectes sapidus (Blue Crab)	3-4 years	shrimp, detritus, decaying fish	0-34 ppt	inhabits estuaries year-round, females seek higher salinity during spawning	Van Den Avyle and Fowler (1984)

Table 3.3: Target ions and retention time windows for GC-NCI-MS-selected ion monitoring (SIM) analyses.

TOX	**Ions (m/z)**	**SIM Window**
Pentachloro-bornanes	275, 273	1
hexachloro-bornanes	309, 307	1, 2
Heptachloro-bornanes	343, 345	1, 2, 3
octachloro-bornanes	377, 379	2, 3, 4
Nonachloro-bornanes	411, 413	3, 4, 5
decachloro-bornanes	445, 447	4, 5
PCB		
dichloro-biphenyl	220	2
trichloro-biphenyl	256	2, 3
tetrachloro-biphenyl	292	2, 3, 4
Pentachloro-biphenyl	326	2, 3, 4, 5
hexachloro-biphenyl	360	4, 5
Heptachloro-biphenyl	396	4, 5
octachloro-biphenyl	430	5, 6
Nonachloro-biphenyl	464	6
Decachloro-biphenyl	498	6

Table 3.4. Toxaphene congener concentrations and lipid content of invertebrates collected from Terry and Dupree Creeks, Brunswick, GA, USA.

CONGENER	INVERTEBRATES (n)				
	Squid (2)	**Crab (2)**	**Shrimp (3)**	**Mussel (2)**	**Oyster (1)**
% Lipid	1.2 (0.9, 1.5)	8.23 (6.33-10.24)	1.25±0.22	1.36 (1.34-1.38)	0.63
P-11	<0.20	ND	<0.20	ND	<0.28
P-12	<0.20	ND	<0.20	ND	<0.28
P-15	9.30 (8.09, 10.51)	7.11 (4.57-9.65)	1.35±0.45	12.74 (9.24-16.23)	<0.28
P-21	<0.20	ND	<0.20	ND	<0.28
P-26	0.87 (0.50, 1.23)	7.37 (5.20-9.53)	2.47±0.47	4.07 (3.13-5.00)	<0.28
P-25	<0.20, 1.28	ND	<0.20	ND	6.96
P-31	<0.20, 0.96	ND	<0.20	ND	<0.28
P-32	<0.20, 1.38	ND	<0.20	ND	6.34
P-38	<0.20	ND	<0.20	ND	<0.28
P-39	<0.20, 0.19	ND	<0.20	ND	<0.28
P-41	0.31 (0.20, 0.42)	2.75 (1.71-3.76)	0.34±0.15	2.92 (1.70-4.13)	1.44
P-40	2.54 (1.72, 3.36)	3.01 (2.03-3.98)	0.67±0.35	21.30 (16.64-25.96)	8.68
P-42	1.69 (1.10, 2.28)	3.83 (2.58-5.07)	0.11±0.01	16.36 (12.54-20.17)	5.82
P-44	<0.20	ND	<0.20	ND	2.51
P-50	0.14 (0.11-0.16)	3.22 (1.46-4.98)	0.86±0.33	3.56 (2.26-4.85)	4.07
P-56	<0.20	1.25 (0.86-1.64)	<0.20	6.91 (6.67-7.15)	<0.28
P-58	<0.20	ND	0.32±0.11	0.35 (0-0.70)	<0.28
P-59	<0.20	ND	<0.20	0.06 (0-0.11)	<0.28
P-62	<0.20, 0.71	ND	<0.20	ND	<0.28
P-63	<0.20, 0.06	0.35 (0.18-0.52)	0.05, <0.20, <0.20	0.46 (0.45-0.47)	0.33
P-69	<0.20	ND	<0.20	ND	<0.28
Hx-Sed	14.93 (11.11, 18.72)	5.28 (4.12-6.43)	3.53±1.05	9.90 (8.68-11.11)	9.60
Hp-Sed	18.37 (13.20, 23.54)	9.34 (6.69-11.98)	2.33±0.64	26.62 (22.10-31.14)	27.76

Table. 3.5. PCB congener concentrations and lipid content of invertebrates collected from Terry and Dupree Creeks, Brunswick, GA, USA.

Congener	INVERTEBRATES (n)				
	Squid (2)	**Crab (2)**	**Shrimp (4)**	**Mussel (2)**	**Oyster (1)**
% Lipid	1.22 (0.90-1.53)	8.23 (6.33-10.24)	1.16±0.20	1.36 (1.34-1.38)	1.26
118	0.20 (0.18-0.22)	4.19 (2.93-5.44)	1.96±1.78	0.38 (0.33-0.42)	0.34
153	0.49 (0.33-0.64)	6.53 (5.77-7.30)	3.25±2.60	1.29 (1.23-1.35)	1.14
105	0.57 (0.54-0.60)	1.21 (1.15-1.27)	0.50±0.04	0.72 (0.62-0.81)	0.69
137	ND	ND	ND	ND	ND
138	0.27 (0.18-0.27)	3.66 (3.21-4.14)	1.05±0.72	0.72 (0.63-0.80)	0.48
187	1.37 (1.03-1.37)	5.55 (3.12-7.98)	0.65±0.09	1.20 (1.18-1.21)	0.74
166	ND	ND	ND	ND	ND
126	ND	ND	ND	ND	ND
128	ND	1.02 (0.89-1.14)	ND	0.16 (0.15-0.16)	0.15
202	0.40 (0.34-0.46)	4.78 (2.50-7.05)	0.19±0.03	0.25 (0.23-0.27)	0.21
204	ND	ND	ND	ND	ND
201	0.08 (0.06-0.09)	1.66 (0.92-2.39)	0.04±0.01	ND	0.16
180	0.21 (0.17-0.24)	3.20 (2.45-3.94)	0.20±0.10	0.1 0.10-0.10)	ND
200	ND	ND	ND	ND	ND
191	ND	ND	ND	ND	ND
170	ND	ND	ND	ND	ND
198	ND	ND	ND	ND	ND
199	0.89 (0.75-1.03)	12.37 (7.76-16.98)	0.58±0.10	0.11 (0.10-0.11)	0.13
196	1.19 (1.00-1.37)	17.63 (13.57-21.68)	0.71±0.08	0.11 (0.09-0.13)	0.07
208	0.25 (0.22-0.28)	4.76 (3.01-6.51)	0.11±0.04	0.01 (0-0.02)	0.01
195/207	0.05 (0.04-0.05)	1.70 (1.20-2.19)	0.03±0.01	0.24 (0.24-0.24)	0.24
194	0.11 (0.10-0.12)	3.60 (2.75-4.44)	0.07±0.01	1.13 (1.06-1.20)	1.00
205	ND	ND	ND	ND	ND
206	1.45 (1.30-1.65)	7.46 (5.98-8.93)	0.79±0.05	0.38 (0.33-0.42)	0.21
209	0.14 (0.11-0.16)	1.89 (1.41-2.37)	0.17±0.04	0.05 (0-0.09)	0.24

Table 3.6. Toxaphene congener concentrations and lipid content of fishes collected from Terry and Dupree Creeks, Brunswick, GA, USA

CONGENER	FISH (n)										
	Flounder (4)	**Anchovy (4)**	**Menhaden (5)**	**Seatrout (3)**	**Mullet (4)**	**Toadfish (2)**	**Kingfish (2)**	**Stingray (1)**	**Mojarra (1)**	**Weakfish (1)**	**Perch (2)**
% Lipid	0.65±0.17	3.92±1.30	1.39±0.30	1.94±0.57	2.40±0.83	0.40±0.04	2.50±1.24	0.44±0.04	4.51	1.70	1.22 (1.06-1.38)
P-11	ND	<0.20	ND	ND	ND	ND	ND	ND	<0.19	<0.20	<0.20
P-12	ND	<0.20	ND	ND	ND	ND	ND	ND	<0.19	<0.28	<0.20
P-15	6.72±2.53	30.01±6.21	25.44±12.59	13.97±6.14	10.28±2.41	0.83±0.20	4.26±2.44	2.54±1.84	33.14	8.55	22.71 (15.59, 29.82)
P-21	ND	<0.20	ND	ND	ND	ND	ND	ND	<0.19	<0.20	<0.20
P-26	1.86±0.54	10.30±2.14	1.27±0.70	4.87±2.62	3.71±1.49	0.53±0.10	1.07±0.42	0.40±0.29	10.11	1.02	12.06 (3.19, 20.98)
P-25	ND	<0.20, <0.20, 10.93, 10.77	ND	ND	ND	ND	ND	ND	<0.19	<0.20	<0.20
P-31	ND	<0.20, <0.20, 6.52, 5.78	ND	ND	ND	ND	ND	ND	<0.19	<0.20	<0.20
P-32	ND	<0.20, 9.47, 8.45, 8.68	ND	ND	ND	ND	ND	ND	<0.19	<0.20	<0.20
P-38	ND	<0.20	ND	ND	ND	ND	ND	ND	<0.19	<0.20	<0.20
P-39	ND	<0.20, 1.73, 1.38, 1.70	ND	ND	ND	ND	ND	ND	<0.19	<0.20	<0.20
P-41	0.63±0.25	2.44±0.12	2.28±1.04	2.04±0.90	4.15±1.36	0.13±0.05	0.22±0.13	0.15±0.12	4.05	22.30	2.95 (0.87, 5.03)
P-40	4.04±1.53	12.51±1.36	8.02±3.42	8.90±4.07	10.20±3.24	0.48±0.23	1.09±0.65	0.93±0.72	13.68	75.91	18.94 (5.65, 32.23)

Table 3.6. Toxaphene congener concentrations and lipid content of fishes collected from Terry and Dupree Creeks, Brunswick, GA, USA. *continued*

CONGENER	FISH (n)										
	Flounder (4)	**Anchovy (4)**	**Menhaden (5)**	**Seatrout (3)**	**Mullet (4)**	**Toadfish (2)**	**Kingfish (2)**	**Stingray (1)**	**Mojarra (1)**	**Weakfish (1)**	**Perch (2)**
% Lipid	0.65±0.17	3.92±1.30	1.39±0.30	1.94±0.57	2.40±0.83	0.40±0.04	2.50±1.24	0.44±0.04	4.51	1.70	1.22 (1.06-1.38)
P-42	3.32±1.24	<0.20, <0.20, 11.24, 8.49	5.81±2.89	11.38±5.27	10.30±3.26	0.25±0.21	1.18±0.62	0.83±0.54	11.53	67.68	18.85 (4.51, 33.18)
P-44	ND	<0.20, 4.66, 6.56, 5.63	ND	ND	ND	ND	ND	ND	<0.19	<0.20	<0.20
P-50	0.91±0.34	4.38±1.24	ND	3.13±2.12	3.02±1.85	ND	ND	ND	4.69	3.35	3.31 (0.97, 5.66)
P-56	ND	<0.20,	ND	ND	ND	0.08±0.05	0.36±0.22	ND	<0.19	<0.20	<0.20
P-58	ND	1.20±0.29	0.32±0.08	0.70±0.34	ND	ND	ND	0.30±0.30	0.67	0.90	0.52 (0.62, 0.42)
P-59	ND	<0.20	ND	ND	ND	ND	0.01±0.01	0.10±0.10	<0.19	<0.20	<0.20
P-62	ND	<0.20	ND	ND	ND	ND	0.11±0.11	0.72±0.72	<0.19	<0.20	<0.20
P-63	0.55±0.44	0.51±0.11	0.29±0.15	0.31±0.17	0.54±0.28	0.01±0.01	0.04±0.03	0.05±0.04	0.81	6.55	0.63 (0.84, <0.20)
P-69	ND	<0.20	ND	ND	ND	ND	ND	ND	<0.19	<0.20	<0.20
Hx-Sed	8.12±2.31	34.32±12.66	13.32±5.84	30.18±11.01	6.28±2.32	1.27±0.76	4.79±3.01	2.36±1.73	13.26	55.01	19.86 (16.58, 23.14)
Hp-Sed	15.90±5.25	74.09±23.06	31.92±14.31	33.92±15.40	14.42±3.01	3.11±1.36	5.24±2.33	5.19±4.00	50.10	179.38	39.81 (32.53, 47.10)

Table 3.7. PCB congener concentrations and lipid content of fishes collected from Terry and Dupree Creeks, Brunswick, GA, USA. *continued*

CONGENER	FISH (n)										
	Flounder (4)	**Anchovy (4)**	**Menhaden (5)**	**Seatrout (3)**	**Mullet (5)**	**Toadfish (3)**	**Kingfish (3)**	**Stingray (3)**	**Mojarra (1)**	**Weakfish (1)**	**Perch (2)**
% Lipid	0.65±0.17	1.80±0.10	1.39±0.30	1.94±0.57	2.40±0.83	0.40±0.04	2.50±1.24	0.44±0.04	4.51	1.70	1.22 (1.06-1.38)
118	0.17±0.10	1.00±0.08	0.35±0.50	1.03±0.38	0.55±0.25	0.19±0.05	0.81±0.58	0.29±0.12	1.23	0.07	0.80 (0.78-0.82)
153	1.66±0.63	3.82±0.88	0.71±0.29	3.11±1.36	1.38±0.36	0.37±0.13	2.35±1.40	0.50±0.25	2.87	1.61	2.81 (2.07-3.54)
105	0.49±0.18	0.88±0.07	0.34±0.15	0.76±0.16	0.43±0.07	0.44±0.03	0.81±0.17	0.68±0.07	0.80	0.47	0.89 (0.81-0.96)
137	0.02±0.01	ND	0.05±0.05	ND	ND	ND	ND	ND	ND	ND	ND
138	0.98±0.27	1.77±0.17	0.39±0.07	1.71±0.65	0.80±0.31	0.33±0.09	0.64±0.34	0.27±0.19	2.60	1.02	1.75 (1.70-1.80
187	3.91±0.58	3.74±1.27	1.14±0.36	4.00±1.54	2.74±0.95	0.81±0.25	6.60±4.14	1.10±0.25	3.01	3.28	2.39 (2.15-2.62)
166	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
126	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
128	0.18±0.09	0.68±0.20	0.22±0.08	0.30±0.06	0.36±0.12	ND	ND	ND	0.51	0.33	0.26 (0.20-0.31)
202	1.07±0.12	1.94±0.44	1.52±0.14	1.14±0.49	2.88±0.87	0.16±0.08	2.26±1.72	0.20±0.06	2.47	2.62	0.32 (0.25-0.38)
204	ND	ND	0.28±0.14	ND	ND	ND	ND	ND	ND	ND	ND
201	0.02±0.01	0.76±0.24	0.34±0.21	0.28±0.11	1.14±0.40	0.05±0.02	0.14±0.03	0.09±0.02	.23	0.46	0.64 (0.60-0.68)

Table 3.6. PCB congener concentrations and lipid content of fishes collected from Terry and Dupree Creeks, Brunswick, GA, USA. *continued*

CONGENER	FISH (n)										
	Flounder (4)	**Anchovy (4)**	**Menhaden (5)**	**Seatrout (3)**	**Mullet (5)**	**Toadfish (3)**	**Kingfish (3)**	**Stingray (3)**	**Mojarra (1)**	**Weakfish (1)**	**Perch (2)**
% Lipid	0.65±0.17	1.80±0.10	1.39±0.30	1.94±0.57	2.40±0.83	0.40±0.04	2.50±1.24	0.44±0.04	4.51	1.70	1.22 (1.06-1.38)
180	0.99±0.20	1.04±0.08	0.28±0.09	1.00±0.41	0.62±0.17	0.21±0.05	1.42±0.96	0.28±0.07	0.72	0.80	0.59 (0.55-0.63)
200	0.02±0.01	ND	0.05±0.03	ND	ND	ND	ND	ND	ND	ND	ND
191	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
170	ND	ND	0.02±0.02	ND	ND	ND	ND	ND	ND	ND	ND
198	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
199	2.54±0.35	5.44±0.82	3.17±1.16	3.32±1.38	6.40±2.05	0.65±0.23	4.96±3.69	1.21±0.23	3.47	6.03	0.85 (0.68-1.02)
196	4.43±0.73	6.17±0.84	3.77±1.39	5.10±2.17	7.35±2.29	0.80±0.27	7.61±5.56	1.65±0.30	5.34	6.71	1.61 (1.43-1.79)
208	0.31±0.12	1.48±0.38	1.71±0.71	0.97±0.35	2.89±0.92	0.19±0.07	2.00±1.41	0.12±0.12	1.07	2.18	0.26 (0.21-0.31)
195/207	0.200.03	0.53±0.13	0.48±0.20	0.21±0.07	0.86±0.29	0.01±0.01	0.35±0.25	0.07±0.02	0.30	0.55	0.49 (0.36-0.62)
194	0.46±0.08	5.31±4.34	0.76±0.31	0.53±0.24	1.61±0.54	0.10±0.03	0.89±0.69	0.18±0.04	1.14	1.27	6.93 (6.07-7.78)
205	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
206	8.75±2.68	5.10±0.89	3.00±1.05	6.33±2.74	4.11±1.25	1.13±0.38	11.73±7.61	2.76±0.39	2.78	3.61	2.92 (2.59-3.25)
209	0.69±0.10	0.67±0.07	0.77±0.29	0.88±0.26	0.92±0.34	0.23±0.05	1.43±0.89	0.39±0.10	0.41	0.72	0.58 (0.56-0.60)

Figure 3.1. Location of sampling site at Brunswick, GA, USA. Samples were taken from the confluence of Terry and Dupree Creeks (indicated by black dot and arrow). Sources of PCBs (LCP Chemicals) and toxaphene (Hercules, Inc.) are marked with stars.

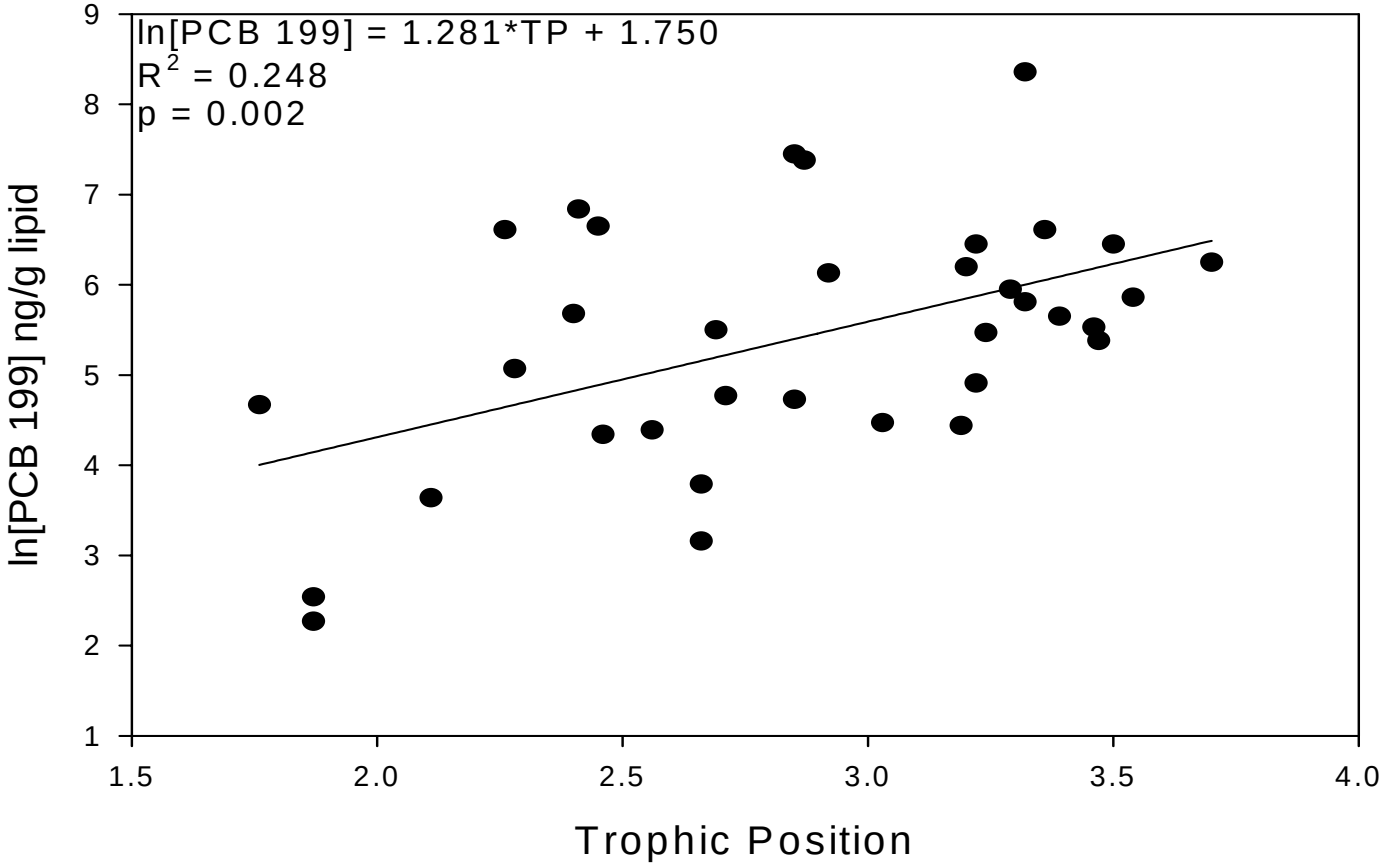

Figure 3.2. Plot of PCB 199 vs. trophic position including all species collected from Brunswick, GA, USA

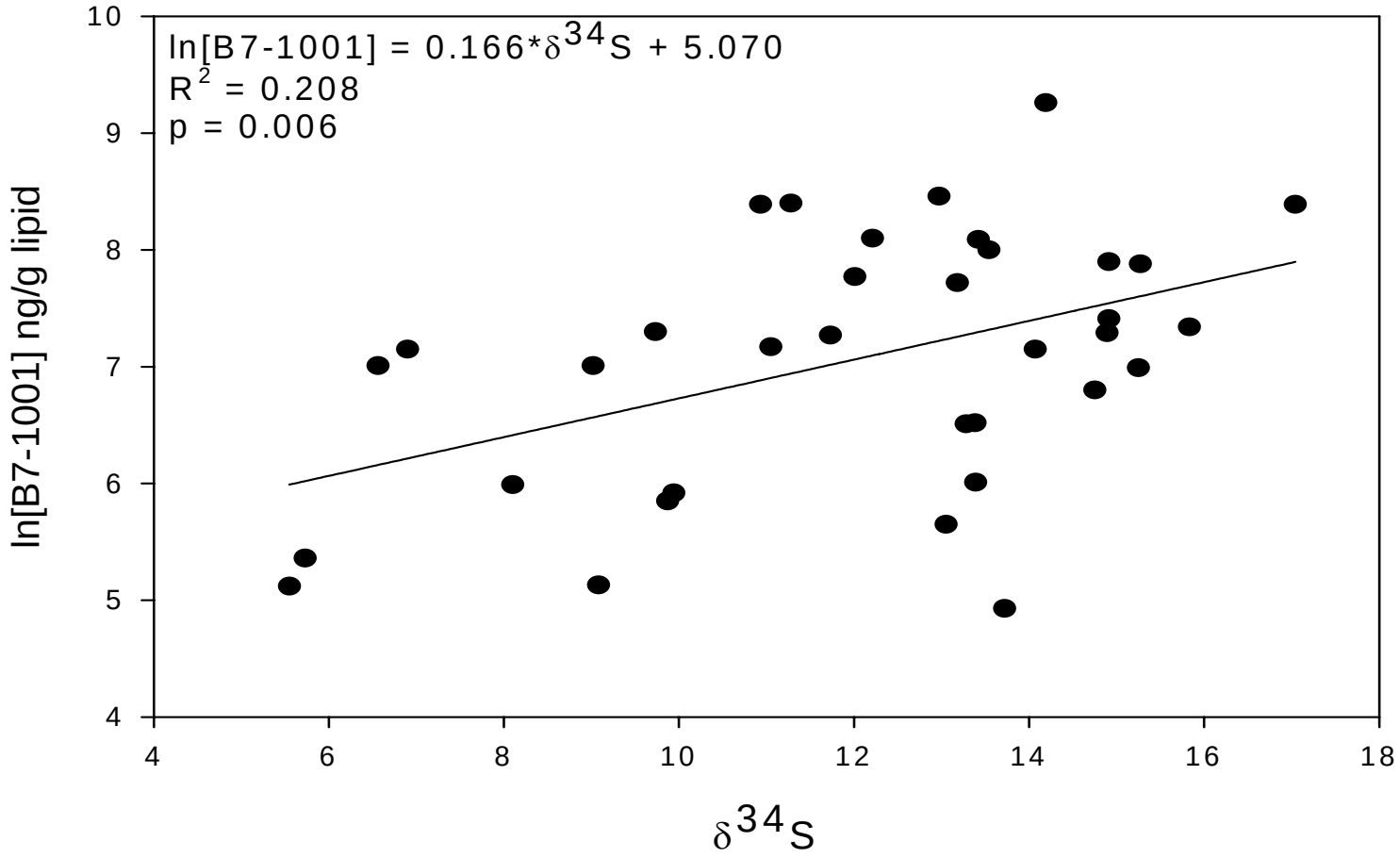

Figure 3.3. Plot of $\delta^{34}S$ vs. concentration of B7-1001 (Hp-Sed) in biota collected from Brunswick, GA, USA.

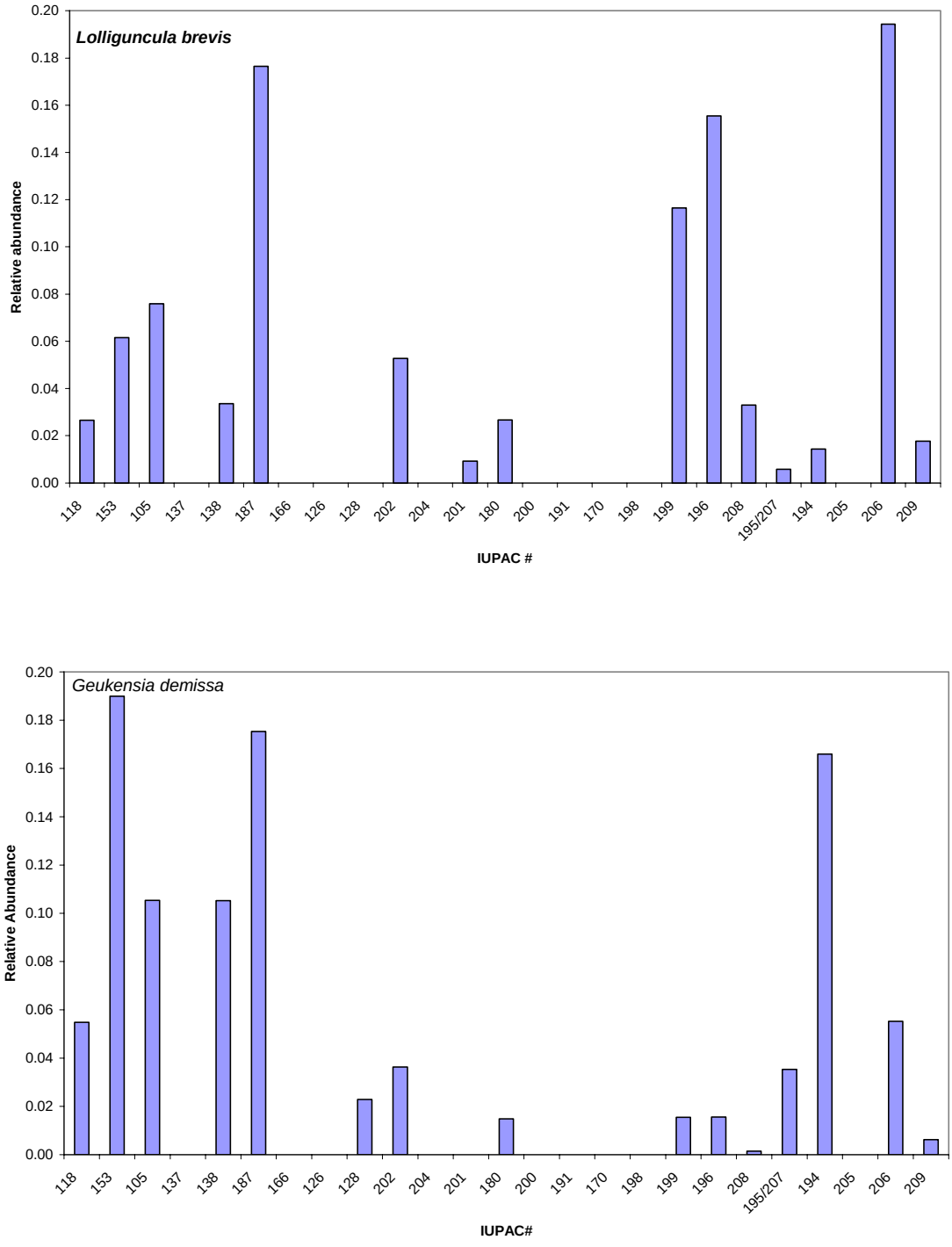

Figure 3.4. Relative abundance of PCB congeners in squid (a) and mussel (b) collected from Brunswick, GA, USA.

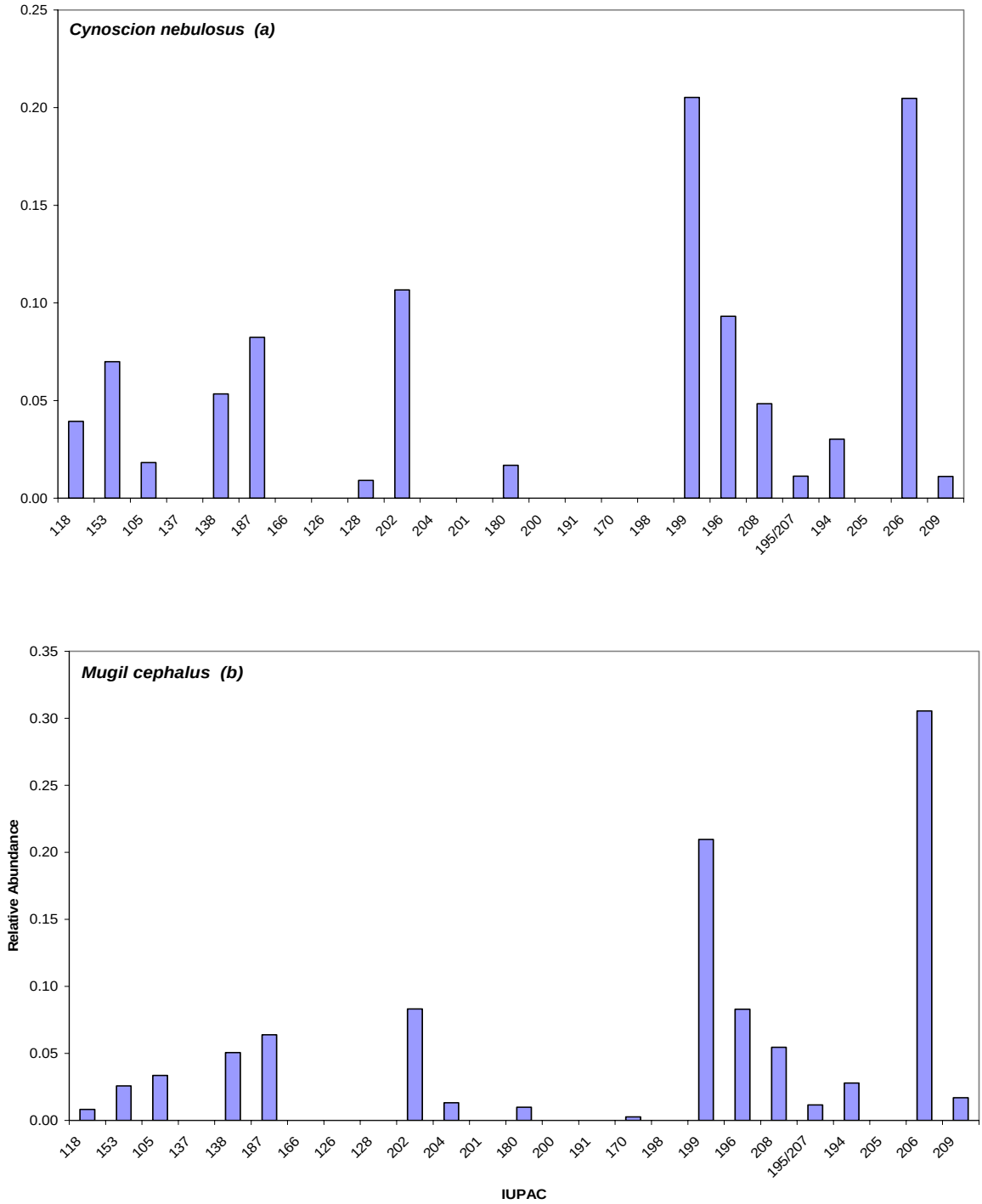

Figure 3.5. Relative abundance of PCB congeners in spotted seatrout (a) and striped mullet (b) collected from Brunswick, GA, USA

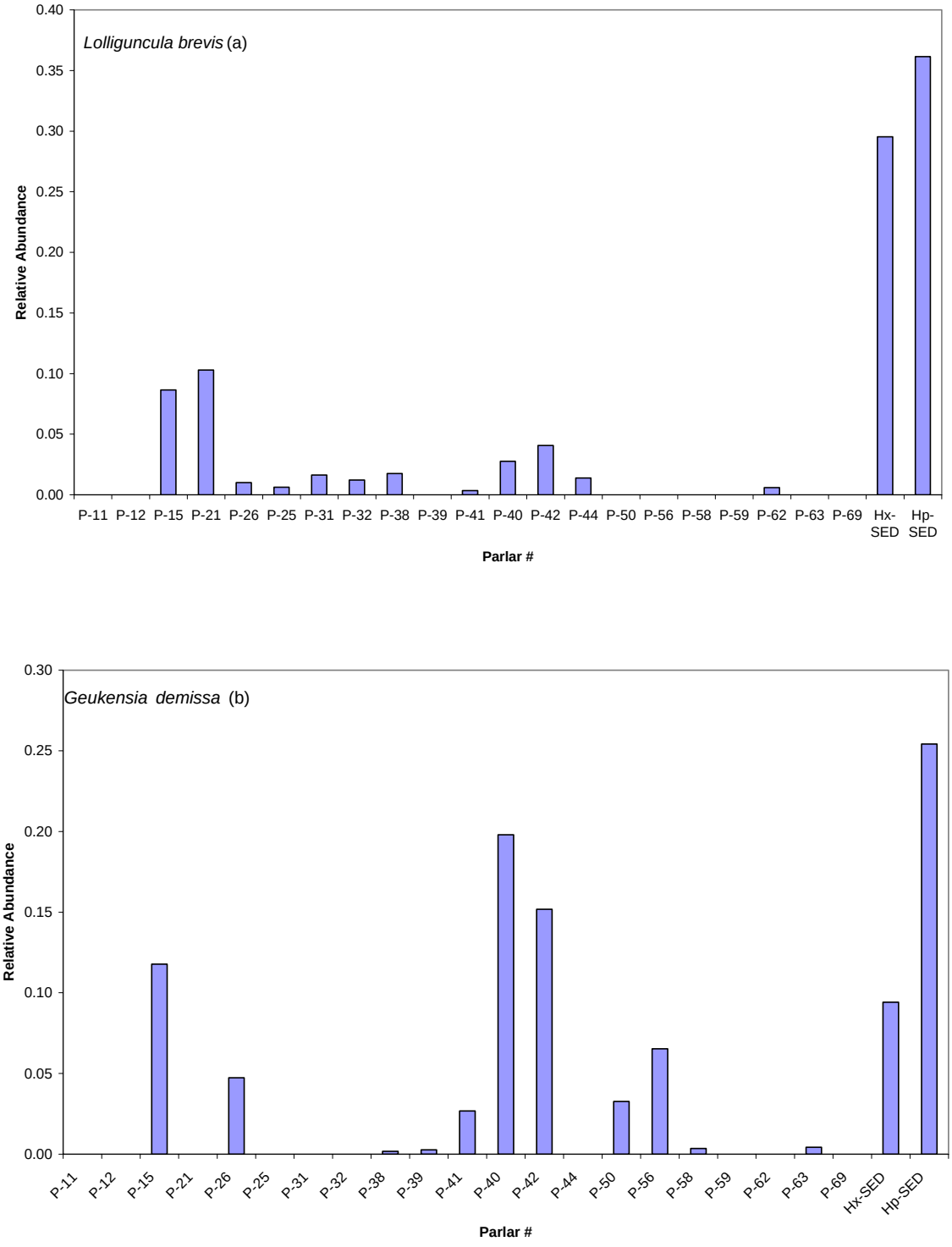

Figure 3.6 Relative abundance of toxaphene congeners in squid (a) and mussel (b) collected from Brunswick, GA, USA

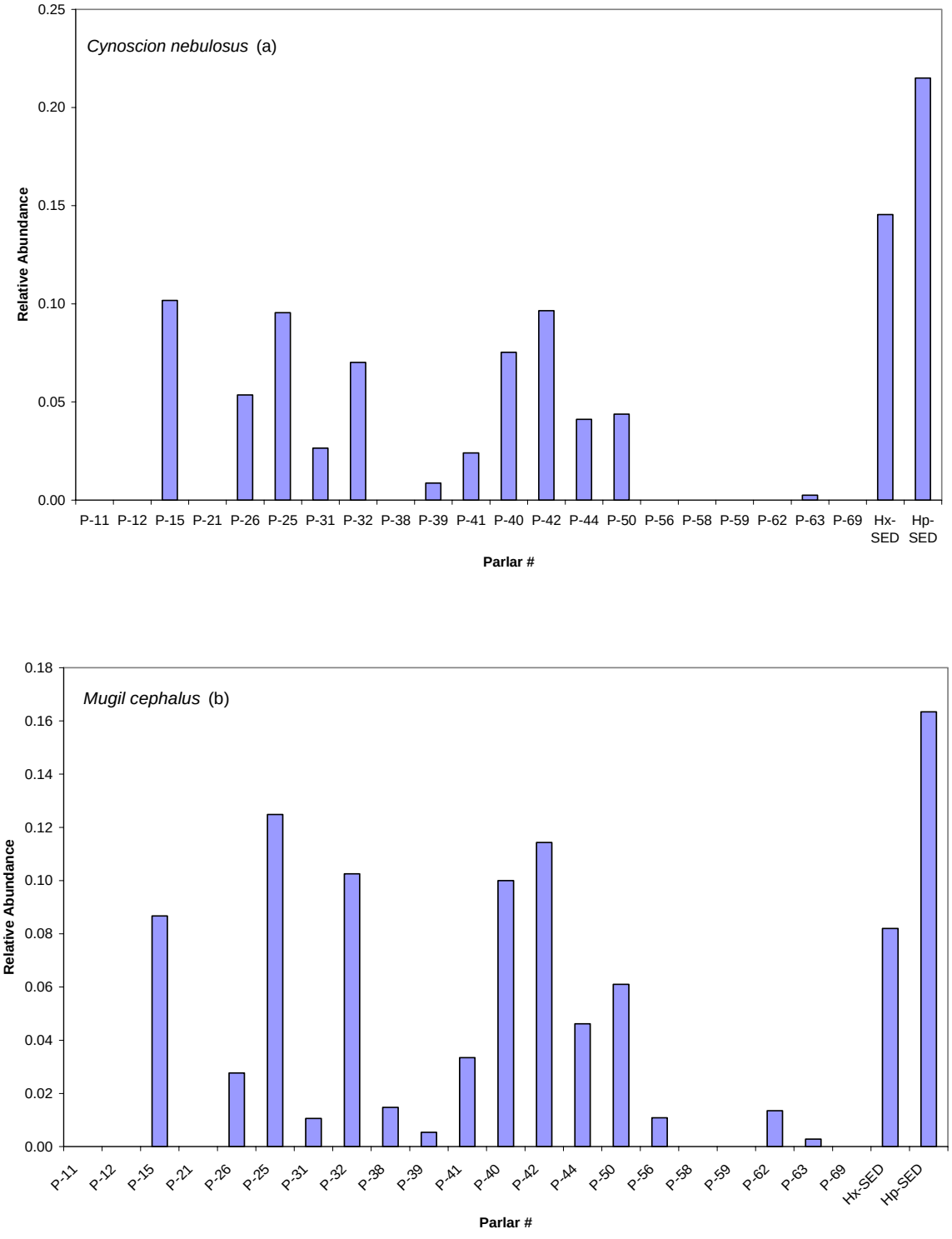

Figure 3.7. Relative abundance of toxaphene congeners in spotted seatrout (a) and striped mullet (b) collected from Brunswick, GA, USA

CHAPTER 4

GENERAL CONCLUSIONS

The aim of this research project was to determine if multiple stable isotopes, as indicators of feeding ecology, could help better assess the trophic transfer of PCBs and toxaphene in a warm temperate estuarine system. Several studies have shown that stable isotopes of nitrogen ($\delta^{15}N$) can be used to quantify trophic transfer of organochloines, such as PCBs and toxaphene, through aquatic food webs (Kidd et al. 1997, Kidd et al. 1998, Kidd et al. 2001, Fisk, et al. 2001, Hop et al. 2002, Ruus et al. 2002, Muir et al. 2004). The majority of studies focusing on the movement of PCBs and toxaphene through entire food webs has been limited to marine and freshwater ecosystems in Arctic and sub-Arctic regions. The employment of $\delta^{15}N$ to assess trophic transfer of PCBs and toxaphene has not been applied to estuaries, specifically those in warm temperate regions.

The estuaries of the southeastern U.S., which are composed primarily of salt marshes, are difficult to study. High tidal ranges, anoxic sediments, and various inputs of organic matter together create an ecosystem with intricate relationships. The complexity of these systems can make the assessment of trophic transfer of PCBs and toxaphene complicated, especially when the estuary contains point sources for both contaminants.

Stable isotopes of carbon and sulfur have been used to study trophodynamics of estuaries in recent years. They have been used to trace organic matter flow (Haines and Montague 1979, Peterson et al. 1986, Peterson and Howarth 1987, Kwak and Zedler 1997, Chanton and Lewis 1999), assess linkages between primary and secondary production (MacAvoy et al. 2000,

Chanton and Lewis 2002, Litvin and Weinstein 2003, Litvin and Weinstein 2004) and determine habitat use of fishes (Fry 2002) in estuaries. Given the wide array of uses in estuarine ecosystem studies it is reasonable to think that these tools could help in the assessment of factors that affect trophic transfer of PCBs and toxaphene in a contaminated estuary.

Stable isotopes of carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$) were able to separate major sources of carbon inputs in Terry and Dupree Creeks. *S. alterniflora* was found to be both enriched in ^{13}C and depleted in ^{34}S, whereas the opposite was found for seston. In a comparable study, Peterson and Howarth (1987) found similar results for seston and *S. alterniflora* in the salt marshes of Sapelo Island, GA, USA, which is approximately 30 km north of Terry and Dupree Creeks. $\delta^{13}C$ values of sediment collected from Terry and Dupree Creeks were the most depleted and had the greatest variability. This suggests that there could be inputs from upland plants into the system, but could also be the result of various microbe-driven reactions altering carbon isotopic signatures in the sediment.

In general, marsh consumers fell within a continuum between seston and *S. alterniflora*. This is also similar to what was reported in Peterson and Howarth (1987). Invertebrate tended to show strong affinity to the pelagic or detrital based food webs. Suspension and filter feeders tended to have isotopic signatures similar to that of seston. On the contrary, deposit feeders had carbon and sulfur signatures that more closely resembled *S. alterniflora*, suggesting a more intimate feeding relationship with the detrial food web rather than pelagic. Higher trophic level consumers (i.e. fishes) tended to converge toward the midpoint between seston and *S. alterniflora*, implying that they perhaps have wider array of food items than invertebrates.

Based on $\delta^{15}N$ values, there were approximately 4 trophic levels measured in this study. Piscivorous fish species such as longnose gar and weakfish (*Cynoscion regalis*) had the highest

calculated trophic position (TP), with values of 3.86 ± 0.12 and 3.7, respectively. Primary producers and sediment had the lowest $\delta^{15}N$ values, resulting in TPs of approximately 1. Invertebrates occupied TP 2 as primary consumers. Most other consumers had TP of about 3, which could imply that these species are omnivorous and feed on similar organisms.

In general, trophic position did not successfully explain PCB and toxaphene concentrations in biota. There were no significant relationships found between toxaphene congeners and trophic position. A few PCB congeners did have a significant relationship with trophic position, but those relationships were weak ($r^2 < 0.26$). This was not totally unexpected given that many of the biota associated with Terry and Dupree Creeks are transient and may feed in various areas distant from the estuary. However, when assessing only resident species only PCB 199 showed a more significant relationship with trophic position ($r^2 = 0.71$, $p < 0.001$).

Overall, carbon and sulfur isotopic signatures did not account for any variability in feeding that might affect the [OC]-TP regression. For PCBs $\delta^{13}C$ and $\delta^{34}S$ only 1-2 % of the variability was explained for most congeners. Just the opposite was found for the toxaphene congener B7-1001. When $\delta^{34}S$ was included in the regression analysis, the multiple regression was found to be significant even though the relationship was weak ($r^2 \sim 0.25$).

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